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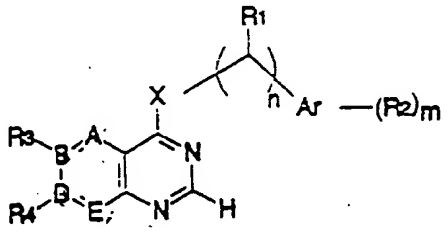
(54) Title: BICYCLIC COMPOUNDS CAPABLE OF INHIBITING TYROSINE KINASES OF THE EPIDERMAL GROWTH FACTOR RECEPTOR FAMILY

(57) Abstract

Epidermal growth factor inhibitors of formula (1), where: at least one, and as many as three of A-E are nitrogen, with the remaining atom(s) carbon, or any two contiguous positions in A-E taken together can be a single heteroatom, N, O or S, in which case one of the two remaining atoms must be carbon, and the other can be either carbon or nitrogen; X = O, S, NH or NR^7, such that R^7 = lower alkyl (1-4 carbon atoms), OH, NH_2, lower alkoxy (1-4 carbon atoms) or lower monoalkylamino (1-4 carbon atoms); n = 0, 1, 2.



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BICYCLIC COMPOUNDS CAPABLE OF INHIBITING TYROSINE KINASES OF THE EPIDERMAL GROWTH FACTOR RECEPTOR FAMILY

Technical Field

The present invention relates to bicyclic heteroaromatic compounds which inhibit the epidermal growth factor receptor and related receptors and, in particular, their tyrosine kinase enzymic activity.

Background Art

Cancer is generally a disease of the intracellular signalling system, or signal transduction mechanism. Cells receive instructions from many extracellular sources, instructing them to either proliferate or not to proliferate. The purpose of the signal transduction system is to receive these and other signals at the cell surface, get them into the cell, and then pass the signals on to the nucleus, the cytoskeleton, and transport and protein synthesis machinery. The most common cause of cancer is a series of defects, either in these proteins, when they

are mutated, or in the regulation of the quantity of the protein in the cell such that it is over or under produced. Most often, there are key lesions in the cell which lead to a constitutive state whereby the cell nucleus receives a signal to proliferate, when this signal is not actually present. This can occur through a variety of mechanisms. Sometimes the cell may start to produce an authentic growth factor for its own receptors when it should not, the so-called autocrine loop mechanism. Mutations to the cell surface receptors, which usually signal into the cell by means of tyrosine kinases, can lead to activation of the kinase in the absence of ligand, and passing of a signal which is not really there. Alternatively, many surface kinases can be overexpressed on the cell surface leading to an inappropriately strong response to a weak signal. There are many levels inside the cell at which mutation or overexpression can lead to the same spurious signal arising in the cell, and there are many other kinds of signalling defect involved in cancer. This invention touches upon cancers which are driven by the three mechanisms just described, and which involve cell surface receptors of the epidermal growth factor receptor tyrosine kinase family (EGFR). This family consists of the EGF receptor (also known as Erb-B1), the Erb-B2 receptor, and its constituitively active oncoprotein mutant Neu, the Erb-B3 receptor and the Erb-B4 receptor. Additionally, other biological processes driven through members of the EGF family of receptors can also be treated by compounds of the invention described below.

The EGFR has as its two most important ligands Epidermal Growth Factor (EGF) and Transforming Growth Factor alpha (TGFalpha). The receptors appear to have only minor functions in adult humans, but are apparently implicated in the disease process of a large portion of all cancers, especially colon and breast cancer. The closely related Erb-B2 Erb-B3 and Erb-B4 receptors have a family of Heregulins as their major ligands, and receptor overexpression and mutation have been unequivocally demonstrated as the major risk factor in poor prognosis breast cancer. Additionally, it has been demonstrated that all four of the members of this family of receptors can form heterodimeric signalling complexes with other members of the family, and that this can lead to synergistic transforming capacity if more than one member of the family is overexpressed in a malignancy. Overexpression of more than one family member has been shown to be relatively common in human malignancies.

The proliferative skin disease psoriasis has no good cure at present. It is often treated by anticancer agents such as methotrexate, which have very serious side effects, and which are not very effective at the toxicity-limited doses which have to be used. It is believed that TGFalpha is the major growth factor overproduced in psoriasis, since 50% of transgenic mice which overexpress TGF alpha develop psoriasis. This suggests that a good inhibitor of EGFR signalling could be used as an antipsoriatic agent, preferably, but not necessarily, by topical dosing.

EGF is a potent mitogen for renal tubule cells. Fourfold increases in both EGF urinary secretion and EGF mRNA have been noted in mice with early stage streptozoicin-induced diabetes. In addition increased expression of the EGFR has been noted in patients with proliferative glomerulonephritis (Roychaudhury et al. *Pathology* **1993**, *25*, 327). The compounds of the current invention should be useful in treating both proliferative glomerulonephritis and diabetes-induced renal disease.

Chronic pancreatitis in patients has been reported to correlate with large increases in expression for both EGFR and TGF alpha. (Korc et al. *Gut* **1994**, *35*, 1468). In patients showing a more severe form of the disease, typified by an enlargement of the head of the pancreas, there was also shown to be overexpression of the erb-B2 receptor (Friess et al. *Ann. Surg.* **1994**, *220*, 183). The compounds of the current invention should prove useful in the treatment of pancreatitis.

In the processes of blastocyte maturation, blastocyte implantation into the uterine endometrium, and other periimplantation events, uterine tissues produce EGF and TGF alpha (Taga *Nippon Sanka Fujinka Gakkai Zasshi* **1992**, *44*, 939), have elevated levels of EGFR (Brown et al. *Endocrinology*, **1989**, *124*, 2882), and may well be induced to produce heparin-binding EGF by the proximity of the developing, but not arrested, blastocyte (Das et al. *Development* **1994**, *120*, 1071). In turn the blastocyte has quite a high level of TGF alpha and EGFR expression (Adamson *Mol. Reprod. Dev.*

1990, *27*, 16). Surgical removal of the submandibular glands, the major site of EGF secretion in the body, and treatment with anti-EGFR monoclonal antibodies both greatly reduce fertility in mice (Tsutsumi et al. *J. Endocrinology* **1993**, *138*, 437), by reducing successful blastocyte implantation. Therefore, compounds of the current invention should prove to have useful contraceptive properties.

PCT patent application Nos. WO92/07844 published May 14, 1992 and WO92/14716 published September 3, 1992 describe 2,4-diaminoquinazoline as potentiators of chemotherapeutic agents in the treatment of cancer.

PCT published application No. WO92/20642 published November 26, 1992 discloses bismono- and bicyclic aryl and heteroaryl compounds which inhibit EGF and/or PDGF receptor tyrosine kinase.

It is an object of the present invention to inhibit the mitogenic effects of epidermal growth factor utilizing an effective amount of bicyclic pyrimidine derivatives, in particular fused heterocyclic pyrimidine derivatives.

It is another object of the present invention to describe bicyclic pyrimidine derivatives, in particular fused heterocyclic pyrimidine derivatives, as inhibitors of the EGF, Erb-B2 and Erb-B4 receptor tyrosine kinases.

It is yet another object of the present invention to describe bicyclic pyrimidine derivatives,

in particular fused heterocyclic pyrimidine derivatives, that are useful at low dosages as inhibitors of EGF-induced mitogenesis. This therefore leads to a further object of compounds having extremely low cytotoxicity.

It is a further object of the present invention to describe bicyclic pyrimidine derivatives, in particular fused heterocyclic pyrimidine derivatives, that are useful in suppressing tumors, especially breast cancers, where mitogenesis is heavily driven by EGFR family members.

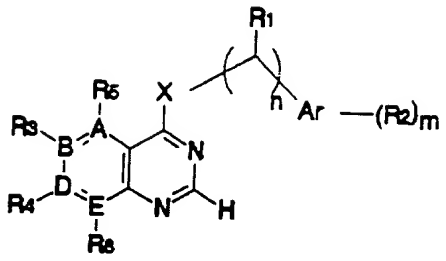
It is another object of the present invention to describe bicyclic pyrimidine derivatives, in particular fused heterocyclic pyrimidine derivatives, that have utility as chronic therapy as inhibitors of EGF-induced responses.

It is another object of the current invention to describe bicyclic pyrimidine derivatives, in particular fused heterocyclic pyrimidine derivatives, that have utility as therapeutic agents against proliferative overgrowth diseases, including but not limited to, synovial pannus invasion in arthritis, vascular restenosis, psoriasis and angiogenesis. The compounds disclosed herein also are useful to treat pancreatitis and kidney disease and as a contraceptive agent.

Summary of the Invention

Described is a method to inhibit epidermal growth factor by treating, with an effective

inhibiting amount, a mammal, in need thereof, a compound of Formula I:



Formula I

wherein at least one, and as many as three of A-E are nitrogen, with the remaining atom(s) carbon, or any two contiguous positions in A-E taken together can be a single heteroatom, N, O or S, in which case one of the two remaining atoms must be carbon, and the other can be either carbon or nitrogen;

X = O, S, NH or NR^7, such that R^7 = lower alkyl (1-4 carbon atoms), OH, NH_2, lower alkoxy (1-4 carbon atoms) or lower monoalkylamino (1-4 carbon atoms);

n = 0, 1, 2;

R^1 = H or lower alkyl (1-4 carbon atoms); if n = 2, R^1 can be independently H or lower alkyl (1-4 carbon atoms) on either linking carbon atom;

R^2 is lower alkyl (1-4 carbon atoms), cycloalkyl (3-8 carbon atoms), lower alkoxy (1-4 carbon atoms), cycloalkoxy (3-8 carbon atoms), nitro, halo (fluoro, chloro, bromo, iodo), lower perfluoroalkyl (1-4 carbon atoms), hydroxy, lower acyloxy (1-4 carbon atoms; -O-C(O)R), amino, lower mono or dialkylamino (1-4 carbon atoms), lower mono or

dicycloalkylamino (3-8 carbon atoms), hydroxymethyl, lower acyl (1-4 carbon atoms; -C(O)R), cyano, lower thioalkyl (1-4 carbon atoms), lower sulfinylalkyl (1-4 carbon atoms), lower sulfonylalkyl (1-4 carbon atoms), thiocycloalkyl (3-8 carbon atoms), sulfinylcycloalkyl (3-8 carbon atoms), sulfonylcycloalkyl (3-8 carbon atoms), sulfonamido, lower mono or dialkylsulfonamido (1-4 carbon atoms), mono or dicycloalkylsulfonamido (3-8 carbon atoms), mercapto, carboxy, carboxamido (-C(O)-NH_2), lower mono or dialkylcarboxamido (1-4 carbon atoms), mono or dicycloalkylcarboxamido (3-8 carbon atoms), lower alkoxycarbonyl (1-4 carbon atoms), cycloalkoxycarbonyl (3-8 carbon atoms), lower alkenyl (2-4 carbon atoms), cycloalkenyl (4-8 carbon atoms), lower alkynyl (2-4 carbon atoms), or two R^2 taken together on contiguous carbon atoms can form a carbocyclic ring of 5-7 members or a monounsaturated 1,3-dioxolanyl, 1,4-dioxanyl, 1,4-dioxepinyl, pyranyl, furanyl, pyrrolidyl, piperidinyl, thiolanyl, oxazolanyl, thiazolanyl, diazolanyl, piperazinyl, morpholino or thiomorpholino ring; and

m = 0-3, wherein Ar is phenyl, thienyl, furanyl, pyrrolyl, pyridyl, pyrimidyl, imidazoyl, pyrazinyl, oxazolyl, thiazolyl, naphthyl, benzothienyl, benzofuranyl, indolyl, quinolinyl, isoquinolinyl and quinazolinyl;

R^3, R^4, R^5 and R^6 are independently, not present, H, lower alkyl (1-4 carbon atoms), cycloalkyl (3-8 carbon atoms), lower alkoxy (1-4 carbon atoms), cycloalkoxy (3-8 carbon atoms), hydroxy, lower acyloxy (1-4 carbon atoms), amino, lower mono or dialkylamino (1-4 carbon atoms), lower mono or dicycloalkylamino (3-8 carbon atoms), lower alkyl (1-4 carbon atoms) or cycloalkyl (3-8 carbon atoms), carbonato (-OC(O)OR)

where the R is lower alkyl of 1 to 4 carbon atoms or cycloalkyl of 3-8 carbon atoms;

or ureido or thioureido or N- or O- linked urethane any one of which is optionally substituted by mono or di-lower alkyl (1-4 carbon atoms) or cycloalkyl (3-8 carbon atoms);

lower thioalkyl (1-4 carbon atoms), thiocycloalkyl (3-8 carbon atoms), mercapto, lower alkenyl (2-4 carbon atoms), hydrazino,N'-lower alkylhydrazino (1-4 carbon atoms), lower acylamino (1-4 carbon atoms), hydroxylamino, lower O-alkylhydroxylamino (1-4 carbon atoms);

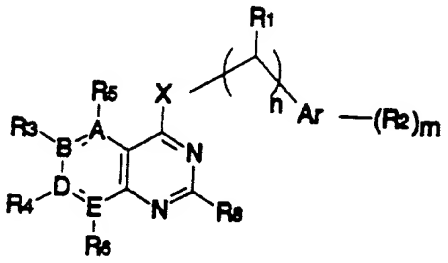
or any two of R^3-R^6 taken together on contiguous carbon atoms can form a carbocyclic ring of 5-7 members or a monounsaturated 1,3-dioxolanyl, 1,4-dioxanyl, 1,4-dioxepinyl, pyranyl, furanyl, pyrrolidyl, piperidinyl, thiolanyl, oxazolanyl, thiazolanyl, diazolanyl, piperazinyl, morpholino or thiomorpholino ring;

any lower alkyl group substituent on any of the substituents in R^3-R^6 which contain such a moiety can be optionally substituted with one or more of hydroxy, amino, lower monoalkylamino, lower dialkylamino, N-pyrrolidyl, N-piperidinyl, N-pyridinium, N-morpholino, N-thiomorpholino or N-piperazino groups;

if one or more of A through E are N, then any of R^3-R^6 on a neighboring C atom to one of the N atoms, cannot be either OH or SH; and

if any of the substituents R^1, R^2, R^3, R^4, R^5 or R^6 contain chiral centers, or in the case of R^1 create chiral centers on the linking atoms, then all stereoisomers thereof both separately and as racemic and/or diastereoisomeric mixtures are included.

Described also is a method to inhibit epidermal growth factor by treating, with an effective inhibiting amount, a mammal, in need thereof, a compound of Formula II:



Formula II

wherein

Ar, n, m, R_1 - R_7 and X are the same as in Formula I;

R^8 is alkyl of from 1-4 carbon atoms or amino or mono or dilower alkyl (1-4 carbon atoms) amino.

The invention is also applicable to the compositions of Formulae I and II with the proviso that at least one of the R^3-R^6 substituents must be taken singly as a substituent other than hydrogen, halo, lower alkyl (1-4 carbon atoms) or lower alkoxy (1-4 carbon atoms), and with the proviso that A, B, D and E must all be taken singly as carbon or nitrogen atoms.

Brief Description Of The Drawings

FIGURE 1 is an effect of Examples 6 and 7 on EGF receptor autophosphorylation in A431 human epidermoid carcinoma;

FIGURE 2 is an effect of Example 8 on EGF receptor autophosphorylation in A431 human epidermoid carcinoma;

FIGURE 3 is a time course for the inhibition of EGF receptor autophosphorylation in A431 by Example 27;

FIGURE 4 is an effect of Example 27 on EGF receptor autophosphorylation in A431 cells;

FIGURE 5 is an inhibition of EGF receptor autophosphorylation in A431 human epidermoid carcinoma by Example 40;

FIGURE 6 is an effect of Example 40 on growth factor-mediated tyrosine phosphorylation in Swiss 3T3;

FIGURE 7 is an effect of Example 40 on growth factor dependent expression of c-jun mRNA in Swiss 3T3 mouse fibroblasts;

FIGURE 8 is an effect of Example 40 on growth factor mediated expression of $p39^{c\text{-}jun}$;

FIGURE 9 is an effect of Example 59 of EGF receptor autophosphorylation in A431 human epidermoid carcinoma;

FIGURE 10 is an effect of Example 60 on EGF receptor autophosphorylation in A431 human epidermoid carcinoma;

FIGURE 11 is an effect of Example 61 on EGF receptor autophosphorylation in A431 human epidermoid carcinoma;

FIGURE 12 is an effect of Example 70 on EGF receptor autophosphorylation in A431 human epidermoid carcinoma;

FIGURE 13 is a chart showing an inhibition of EGF receptor tyrosine kinase by Example 27;

FIGURE 14 is a graph showing an effect of Example 40 on growth factor-mediated mitogenesis in Swiss 3T3 murine fibroblasts;

FIGURE 15 is a photograph of an NIH 3T3 mouse fibroblast line, transfected with the human EGFR gene showing a normal flattened morphology;

FIGURE 16 is a photograph of the same cell line treated with 100 ng/mL of EGF showing a typical spindly transformed morphology; and

FIGURE 17 is a photograph of the same cell line in the presence of both 100 ng/mL of EGF and 5 μm of Example 27 showing the morphology reverted from the transformed type back to the normal type.

Description of Preferred Embodiments

1. A preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, B, D & E carbon,

with A nitrogen and R^3 or R^4 H, with the other one lower alkoxy or halogen.

2. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, B, D & E carbon, with A nitrogen and R^3 or R^4 H, with the other one amino.

3. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, B, D & E carbon, with A nitrogen and R^3 or R^4 H, with the other one lower mono or dialkylamino.

4. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, B, D & E carbon, with A nitrogen and R^3 or R^4 H, with the other one hydrazino.

5. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, B, D & E carbon, with A nitrogen and R^3 or R^4 H, with the other one lower alkyl.

6. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, B, D & E carbon, with A nitrogen and R^3 and R^4 lower alkoxy.

7. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the

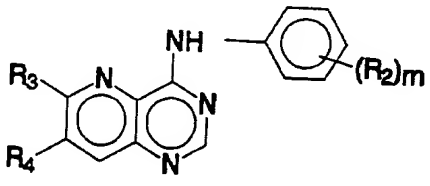
aromatic ring phenyl optionally substituted, B, D & E carbon, with A nitrogen and R^3 and R^4 lower alkyl.

8. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, B, D & E carbon, with A nitrogen, and R^3 or R^4 amino, with the other one lower alkoxy.

9. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, B, D & E carbon, with A nitrogen, and R^3 or R^4 lower mono or dialkylamino, with the other one lower alkoxy.

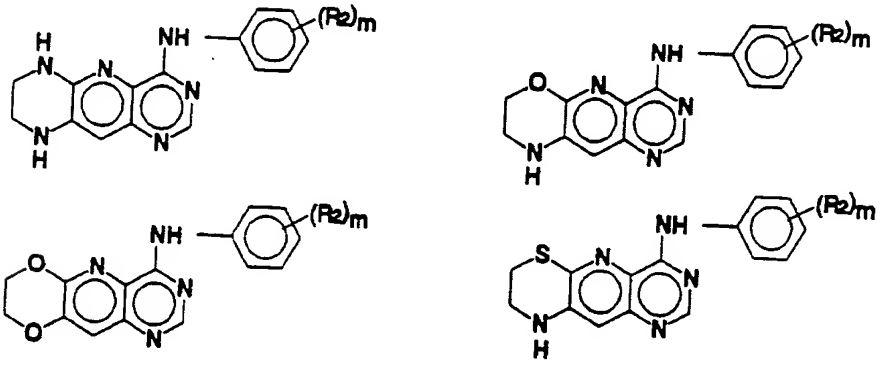
10. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, B, D & E carbon, with A nitrogen and R^3 lower mono or dialkylamino, with R^4 hydroxy.

A suitable ring structure for groups 1-10 is:



11. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, B, D & E

carbon, with A nitrogen, and R^3 and R^4 taken together are dioxymethylene, dioxyethylene, 2,3-fused piperazine, 2,3-fused morpholine or 2,3-fused thiomorpholine. Suitable ring structures are:



12. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, D & E carbon, with B nitrogen and R^4 lower alkoxy or halogen.

13. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, D & E carbon, with B nitrogen and R^4 amino.

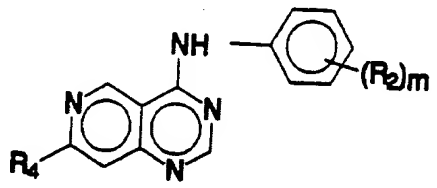
14. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, D & E carbon, with B nitrogen and R^4 lower mono or dialkylamino.

15. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the

aromatic ring phenyl optionally substituted, A, D & E carbon, with B nitrogen and R^4 hydrazino.

16. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, C & E carbon, with B nitrogen and R^4 lower alkyl.

A suitable ring structure for groups 12-16 is:



17. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & E carbon, with D nitrogen and R^3 lower alkoxy or halogen.

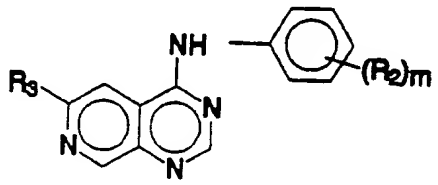
18. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & E carbon, with D nitrogen and R^3 amino.

19. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & E carbon, with D nitrogen and R^3 lower mono or dialkylamino.

20. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & E carbon, with D nitrogen and R^3 hydrazino.

21. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & E carbon, with D nitrogen and R^3 lower alkyl.

A suitable ring structure for groups 17-21 is:



22. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & D carbon, with E nitrogen and R^3 or R^4 H, with the other one lower alkoxy.

23. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & D carbon, with E nitrogen and R^3 or R^4 H, with the other one amino.

24. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the

aromatic ring phenyl optionally substituted, A, B & D carbon, with E nitrogen and R^3 or R^4 H, with the other one lower mono or dialkylamino.

25. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & D carbon, with E nitrogen and R^3 or R^4 H, with the other one hydrazino.

26. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & D carbon, with E nitrogen and R^3 or R^4 H, with the other one lower alkyl.

27. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & D carbon, with E nitrogen and R^3 and R^4 lower alkoxy.

28. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & D carbon, with E nitrogen and R^3 and R^4 lower alkyl.

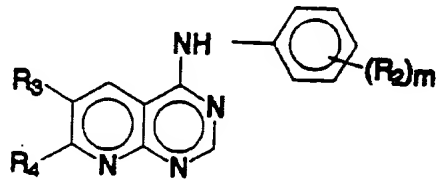
29. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & D carbon, with E nitrogen, and R^3 or R^4 amino, with the other one lower alkoxy.

30. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the

aromatic ring phenyl optionally substituted, A, B & D carbon, with E nitrogen, and R^3 or R^4 lower mono or dialkylamino, with the other one lower alkoxy.

31. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & D carbon, with E nitrogen and R^4 lower mono or dialkylamino, with R^3 hydroxy.

A suitable ring structure for groups 22-31 is:



32. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & D carbon, with E nitrogen, and R^3 and R^4 taken together are dioxymethylene, dioxyethylene, 2,3-fused piperazine, 2,3-fused morpholine or 2,3-fused thiomorpholine.

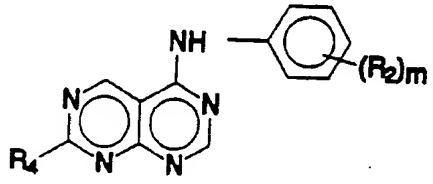
33. Another preferred form of the invention has X = NH, n = 0, the aromatic ring phenyl optionally substituted, A & D carbon, with B and E nitrogen and R^4 lower alkoxy.

34. Another preferred form of the invention has X = NH, n = 0, the aromatic ring phenyl optionally substituted, A & D carbon, with B and E nitrogen and R^4 lower mono or dialkylamino.

35. Another preferred form of the invention has X = NH, n = 0, the aromatic ring phenyl optionally substituted, A & D carbon, with B and E nitrogen and R^4 amino.

36. Another preferred form of the invention has X = NH, n = 0, the aromatic ring phenyl optionally substituted, A & D carbon, with B and E nitrogen and R^4 hydrazino.

A suitable ring structure for groups 33-36 is:



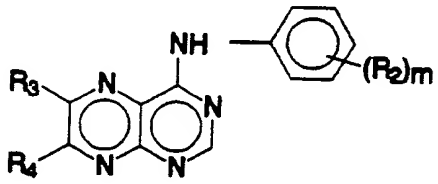
37. Another preferred form of the invention has X = NH, n = 0, the aromatic ring phenyl optionally substituted, B & D carbon, with A and E nitrogen and R^3 and R^4 lower alkoxy.

38. Another preferred form of the invention has X = NH, n = 0, the aromatic ring phenyl optionally substituted, B & D carbon, with A and E nitrogen and R^3 and R^4 lower mono or dialkylamino.

39. Another preferred form of the invention has X = NH, n = 0, the aromatic ring phenyl optionally substituted, B & D carbon, with A and E nitrogen and R^3 or R^4 lower alkoxy, with the other lower mono or dialkylamino.

40. Another preferred form of the invention has X = NH, n = 0, the aromatic ring phenyl optionally substituted, B & D carbon, with A and E nitrogen and R^3 and R^4 taken together are ethylenedioxy, 2,3-fused piperazine, 2,3-fused morpholine or 2,3-fused thiomorpholine.

A suitable ring structure for groups 37-40 is:



41. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, and either A and B taken together are a sulfur atom, with D & E carbon, or A & B are carbon with D and E taken together as a sulfur atom, with R^4 or R^3 H, lower alkyl, lower alkoxy, amino, or lower mono or dialkylamino.

42. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the

aromatic ring phenyl optionally substituted, and either A and B taken together are an oxygen atom, with D & E carbon, or A & B are carbon with D and E taken together as an oxygen atom, with R^4 or R^3 H, lower alkyl, lower alkoxy, amino, or lower mono or dialkylamino.

43. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, and either A and B taken together are a nitrogen atom, with D & E carbon, or A & B are carbon with D and E taken together as a nitrogen atom, with R^4 or R^3 H, lower alkyl, lower alkoxy, amino, or lower mono or dialkylamino.

44. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, and either A and B taken together are a sulfur atom with D carbon and E nitrogen, or D and E taken together are a sulfur atom, and A is nitrogen and B is carbon, with $R^{3/4}$ H, lower alkyl, lower alkoxy, amino, or lower mono or dialkylamino.

45. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, and either A and B taken together are an oxygen atom with D carbon and E nitrogen, or D and E taken together are an oxygen atom, and A is nitrogen and B is carbon, with $R^{3/4}$ H, lower alkyl, lower alkoxy, amino, or lower mono or dialkylamino.

46. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A and B taken together are a nitrogen atom, and D is carbon and E is nitrogen, with $R^{3/6}$ H, or lower alkyl, and R^4 H, lower alkyl, lower alkoxy, amino, or lower mono or dialkylamino.

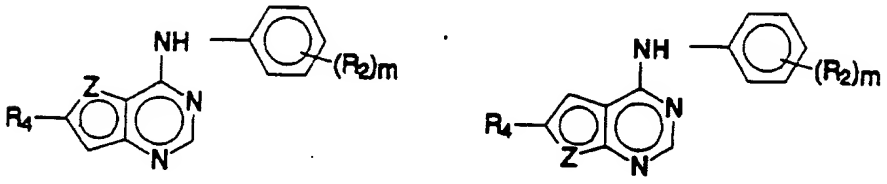
47. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, and either A and B taken together are an oxygen atom with D nitrogen and E carbon, or A and B taken together are a carbon atom with D nitrogen and E oxygen, with $R^{3/6}$ H, lower alkyl, lower alkoxy, amino, or lower mono or dialkylamino.

48. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, and either A and B taken together are a sulfur atom with D nitrogen and E carbon, or A and B taken together are a carbon atom with D nitrogen and E sulfur, with $R^{3/6}$ H, lower alkyl, lower alkoxy, amino, or lower mono or dialkylamino.

49. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, and either A and B taken together are a nitrogen atom with D nitrogen and E carbon, or A and B taken together are a carbon atom with D and E nitrogen atoms, with $R^{3/6}$ H or lower alkyl if on nitrogen, or H, lower alkyl,

lower alkoxy, amino, or lower mono or dialkylamino if on carbon.

Other suitable ring structures are:



Where Z = nitrogen, oxygen or sulfur

The compounds of the present invention are prepared according to a number of alternative reaction sequences.

Preparative Routes to Compounds of the Invention

Scheme 1 -
Route for Preferred Groups 1-5, R^4 = H

Displacement of the 2-chloro of 2,6-dichloro-3-nitropyridine is carried out by cuprous cyanide in NMP. Displacement of the second chlorine of this nitrile by fluoride at this step can be advantageous. This is followed by a mild reduction of the nitro group, under conditions where the halogen is not hydrogenolysed. Hydrolysis of the nitrile followed by orthoformate cyclization, and Vilsmeier-type chlorination will give the dihalo-pyridopyrimidine. Displacement of the more reactive 4-chlorine with an appropriate amine is followed by displacement of the 6-halogen with the appropriate nucleophile, ammonia, lower alkylamine, hydrazine, methoxide, to form the final products. (NMP is a solvent, N-methyl-2-pyrrolidone).

1. A preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, B, D & E carbon, with A nitrogen and R^3 or R^4 H, with the other one lower alkoxy or halogen.

2. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, B, D & E carbon, with A nitrogen and R^3 or R^4 H, with the other one amino.

3. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, B, D & E carbon, with A nitrogen and R^3 or R^4 H, with the other one lower mono or dialkylamino.

4. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, B, D & E carbon, with A nitrogen and R^3 or R^4 H, with the other one hydrazino.

5. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, B, D & E carbon, with A nitrogen and R^3 or R^4 H, with the other one lower alkyl.

Scheme 2 -
Route to Preferred Groups 1-5, R^3 = H

Displacement of chlorine from 2-chloro-3,5-dinitropyridine is accomplished with CuCN in NMP.

Reduction of the nitro groups to amines is followed by hydrolysis of the nitrile to an amide. This is cyclized to the pyrimidone with orthoformate, which is converted to the chloride by $POCl_3$ or possibly turned into the thiomethyl derivative by treatment with phosphorus pentasulfide followed by MeI and a mild base. Displacement with the appropriate amine gives the desired 7-amino compound. The amine functionality can be reductively alkylated or activated by diazotisation of the amino group under acidic or basic conditions, followed by a reduction to the hydrazide, or conversion into a lower alkyl ether, or to a halogen followed by a cuprate or Stille coupling by methods familiar to those skilled in the art. Alternatively, the amine can be reductively aminated, or acylated and reduced to form the alkylamino side chain.

Scheme 3 -
Route to Preferred Groups 6 and 8-10 where R^4 = RO

The known metalation of 2,6-difluoropyridine is exploited twice. LDA treatment followed by a borate/hydrogen peroxide introduces the 3-hydroxy substituent. If the pyridine undergoes the 2nd metalation at the 4 position, the alcohol can be protected as a TIPS (triisopropyl silyl) ether, which will force the second metalation to the 5-position. Alternative nitrations may be used, such as converting the lithium intermediate to a stannane and treatment with tetranitromethane, or the use of NO_2BF_4 (nitronium tetrafluoroborate). The C_1 displacement may be effected by cuprous cyanide or other sources of cyanide ion. After nitrile hydrolysis and nitro group reduction, ethyl orthoformate may be used instead of

formamide for the cyclization, and it may be that some cyclizations will require displacement of F by MeS prior to the reaction. The 4-position is activated by chlorination, and the sidechain amine is then introduced. The final displacement can be by alkoxide or amine nucleophiles to generate the various dialkoxy and amino-alkoxy species, and the appropriate use of R can allow the 7-hydroxyl group to be unmasked at the end of the synthesis. (LDA means lithium diisopropyl amide).

6. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, B, D & E carbon, with A nitrogen and R^3 and R^4 lower alkoxy.

8. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, B, D & E carbon, with A nitrogen, and R^3 or R^4 amino, with the other one lower alkoxy.

9. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, B, D & E carbon, with A nitrogen, and R^3 or R^4 lower mono or dialkylamino, with the other one lower alkoxy.

10. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, B, D & E carbon, with A nitrogen and R^3 lower mono or dialkylamino, with R^4 hydroxy.

Scheme 4 - Route to Preferred Group 7

Use of the 6-alkylquinaldic acid followed by ionic bromination under forcing conditions gives an anhydride, which is opened with ammonia, recyclized to the imide, and then the Hoffman degradation occurs at the less active carbonyl. Cyclization and ring side chain addition in the normal manner is followed by a Stille coupling to introduce the R^4 alkyl group. At this step alkenyl or aryl substituents could also be introduced using this coupling technology.

7. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, B, D & E carbon, with A nitrogen and R^3 and R^4 lower alkyl.

Scheme 5 - Route to Preferred Groups 8, 9, R^3 = OR

Dinitration of 2,6-dihydroxypyridine is followed by conversion to the very reactive dichlorocompound. The dinitrodichloropyridine is singly displaced by cuprous cyanide in NMP, and then the compound is reduced under mild conditions to the diamine. The nitrile is hydrolysed to the amide, which can then be cyclized to the pyridopyrimidone, which is 4-chlorinated in the usual fashion. Displacement of the more reactive chlorine with the 4-sidechain is followed by displacement of the 6-chlorine with alkoxid. For group 9, the amine should be alkylated appropriately by methods familiar to one skilled in the art.

Scheme 6 - Route to Preferred Group 11

Compounds of preferred group 11 are specialized cases of preferred groups 6, 8, 9 and 10, where R^3 and R^4 are cyclized together. They can be made using the same routes as those described for the preferred groups, with minor modifications, which will be obvious to one skilled in the art. For example vicinally substituted alkoxy amino compounds can be dealkylated, and the corresponding vicinal aminoalcohols can be bisalkylated with an appropriate dihaloalkane.

11. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, B, D & E carbon, with A nitrogen, and R^3 and R^4 taken together are dioxymethylene, dioxyethylene, 2,3-fused piperazine, 2,3-fused morpholine or 2,3-fused thiomorpholine.

Scheme 7 - Route for Preferred Groups 12-16

2,4-Diamino-5-cyanopyridine can be cyclized directly to many 4-benzylaminopyridopyrimidine derivatives by treatment with the benzylamine and formic acid at high temperature. For less nucleophilic amines 2,4-diamino-5-cyanopyridine is converted via ethyl orthoformate/acetic anhydride treatment, followed by cyclization with hydrosulfide ion in anhydrous conditions, to give 7-amino-4-thiono-3H-pyrido[4,3-d]pyrimidine. S-Alkylation and displacement with an appropriate amine gives the desired product. If R^4 is not amino, the amine can be

acylated, or reductively alkylated. Alternatively 2,4-diamino-5-cyanopyridine can be hydrolysed to the corresponding amide, and this species can be cyclized to 7-amino-4-oxo-3H-pyrido[4,3-d]pyrimidine with orthoformate. Diazotization of the 7-amine and replacement with fluorine allows for introduction of other amine and alkoxide nucleophiles at the end of the synthesis after the C4 substituent has been introduced in the usual manner. Diazotization and replacement of the amine with bromide allows for Stille couplings at the 7-position.

12. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, D & E carbon, with B nitrogen and R^4 lower alkoxy or halogen.

13. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, D & E carbon, with B nitrogen and R^4 amino.

14. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, D & E carbon, with B nitrogen and R^4 lower mono or dialkylamino.

15. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, D & E carbon, with B nitrogen and R^4 hydrazino.

16. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, C & E carbon, with B nitrogen and R^4 lower alkyl.

Scheme 8 - Route for Preferred Groups 17-21

2-Chloro-5-nitropyridine is converted to the corresponding 2-fluorocompound by KF in DMSO. Reduction of the nitro group followed by treatment with Boc anhydride gives the Bocamino derivative, which can be metalated and carboxylated at the 4-position. Removal of the Boc with TFA and cyclization of the pyrimidone ring with formamide gives 6-fluoro-4-oxo-3H-pyrido[3,4-d]pyrimidine. This is 4-chlorinated in the usual manner and the 4-sidechain is introduced via displacement with an appropriate amine. Displacement of the 6-fluorine with appropriate nucleophiles leads to various different final products. If the fluorine is displaced by thiomethoxide, that in turn can be displaced by alkyl groups in Ni-catalyzed Grignard displacements.

17. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & E carbon, with D nitrogen and R^3 lower alkoxy or halogen.

18. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & E carbon, with D nitrogen and R^3 amino.

19. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & E carbon, with D nitrogen and R^3 lower mono or dialkylamino.

20. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & E carbon, with D nitrogen and R^3 hydrazino.

21. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & E carbon, with D nitrogen and R^3 lower alkyl.

Scheme 9 - Route to Preferred Groups 22-26, R^4 = H

Nitration of 2-methoxynicotinic acid is followed by displacement of the activated methoxy group and cyclization of the pyrimidone ring, possibly all in one step with formamidine, or alternatively in two steps with ammonia followed by cyclization with a formamide equivalent. The carbonyl is converted to the chloride and displaced with the sidechain in the usual fashion, and the nitro group is then selectively reduced to amino. This can be alkylated, acylated or diazotized. The diazo compound can be converted to hydroxy or to the bromide or iodide, and these latter can undergo a Stille coupling to introduce lower alkyl, alkenyl, aryl, etc. at R^3.

22. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the

aromatic ring phenyl optionally substituted, A, B & D carbon, with E nitrogen and R^3 or R^4 H, with the other one lower alkoxy.

23. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & D carbon, with E nitrogen and R^3 or R^4 H, with the other one amino.

24. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & D carbon, with E nitrogen and R^3 or R^4 H, with the other one lower mono or dialkylamino.

25. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & D carbon, with E nitrogen and R^3 or R^4 H, with the other one hydrazino.

26. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & D carbon, with E nitrogen and R^3 or R^4 H, with the other one lower alkyl.

Scheme 10 - Route to Preferred Groups 22-26, R^3 = H

This route uses the known metalation and carboxylation of 2,6-difluoropyridine, followed by displacement of the 2-fluoro substituent. Cyclization of the pyrimidone ring with formamide, followed by

conversion of the carbonyl into chloride in a normal manner gives a chlorofluoropyridopyrimidine. The ar(alk)ylamino sidechain is introduced by displacement of the more reactive pyrimidine chlorine, and the R^4 substituent is then introduced by fluoride displacement. The introduction of alkyl utilizes displacement of F by alkoxide, later ether cleavage to the pyridone, O-triflation and Stille coupling.

Scheme 11 -
Route to Preferred Groups 27 and 29-31, R^3 = RO

This scheme relies on the metalation of 2,6-difluoropyridine similarly to scheme 10. The first metalation is used to introduce oxygen, and the second to introduce the carboxylic acid. If required to force the second metalation to the 5-position the oxygen may be protected as the very bulky TIPS ether, and stronger bases than LDA may be required. Ammonia is introduced at the 2-position under high temperature and pressure, and the pyridone ring is cyclized, and activated at the 4-position in the usual manner and then displaced with the 4-position sidechain. Displacement of the 7-fluoro substituent with an appropriate nucleophile, followed by conversions as described in previous schemes finishes the synthesis.

27. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & D carbon, with E nitrogen and R^3 and R^4 lower alkoxy.

29. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & D

carbon, with E nitrogen, and R^3 or R^4 amino, with the other one lower alkoxy.

30. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & D carbon, with E nitrogen, and R^3 or R^4 lower mono or dialkylamino, with the other one lower alkoxy.

31. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & D carbon, with E nitrogen and R^4 lower mono or dialkylamino, with R^3 hydroxy.

Scheme 12 - Route to Preferred Group 28

5-Bromo-2,6-difluoronicotinic acid is prepared from 2,6-difluoropyridine by successive lithiations using LDA. The 5-position is alkylated via a Stille coupling, and the pyrimidone ring is cyclized on in two steps. The 4-substituent is introduced in the usual fashion and the 7-fluoro group is displaced with thiomethoxide. This thioether in turn is displaced by a Grignard agent in the presence of a nickel salt catalyst. Again use of appropriate organometallic reagents in the Stille and Grignard couplings could lead to alkenyl, alkynyl and aryl substituents at R^3 and R^4.

28. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & D carbon, with E nitrogen and R^3 and R^4 lower alkyl.

Scheme 13 - Route to Preferred Groups 29 and 30, R^4 = RO

Nitration of the commercially available dichloronicotinic acid is followed by a selective displacement of the more reactive Cl under mild conditions, followed by a more forcing displacement of the other Cl, in the appropriate order. The resulting 6-alkoxy-2-amino-5-nitronicotinic acid is cyclized to the pyrimidone, and the 4-carbonyl is converted to a chloride and displaced in the usual fashion with an appropriate amine to give the 4-amino-7-alkoxy-6-nitropyrido[2,3-d]pyrimidine. Reduction of the nitro group, followed by any desired alkylation or acylation gives the desired compounds.

Scheme 14 - Route to Preferred Group 32

Compounds of group 32 are specialized cases of preferred groups 27, 29, 30 and 31, where R^3 and R^4 are cyclized together. They can be made using the same routes as those described for these preferred groups with minor modifications. For example, vicinally substituted alkoxy amino compounds can be dealkylated, and the corresponding vicinal amino alcohols can be bisalkylated with an appropriate dihaloalkane. Piperazines can be made by the route shown in Scheme 13, provided that a suitable amine nucleophile is used to displace the 6-chloro substituent instead of an alkoxide.

32. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, A, B & D carbon, with E nitrogen, and R^3 and R^4 taken together are dioxymethylene, dioxyethylene, 2,3-fused

piperazine, 2,3-fused morpholine or 2,3-fused thiomorpholine.

Scheme 15 - Route to Preferred Groups 33-36

Reaction of a suitable S-alkylisothiouronium salt with methoxymethylidine malononitrile yields a fully functionalized pyrimide precursor. The initially formed pyrimidine can have the SEt displaced by R^4 either before or after the nitrile hydrolysis, if displacement or oxidation prove problematic later. Displacement of the SEt group can also be achieved without an oxidation to activate the sulfur. Cyclization of the second pyrimidine ring is followed by activation of the 4-carbonyl by thiation and alkylation. Even if the 7-thio group has not been displaced at this point, introduction of the 4-amino sidechain occurs preferentially.

33. Another preferred form of the invention has X = NH, n = 0, the aromatic ring phenyl optionally substituted, A & D carbon, with B and E nitrogen and R^4 lower alkoxy.

34. Another preferred form of the invention has X = NH, n = 0, the aromatic ring phenyl optionally substituted, A & D carbon, with B and E nitrogen and R^4 lower mono or dialkylamino.

35. Another preferred form of the invention has X = NH, n = 0, the aromatic ring phenyl optionally substituted, A & D carbon, with B and E nitrogen and R^4 amino.

36. Another preferred form of the invention has X = NH, n = 0, the aromatic ring phenyl optionally substituted, A & D carbon, with B and E nitrogen and R^4 hydrazino.

Scheme 16 - Route to Preferred Groups 37-40

The pterine nucleus is made by well-established procedure. For group 37, the pterindione intermediate can be O-alkylated, and for it, and the other groups, the pterindione can be converted to the trichloropterin, and selective displacements can be carried out on the halogens in an order appropriate to give the desired compound.

37. Another preferred form of the invention has X = NH, n = 0, the aromatic ring phenyl optionally substituted, B & D carbon, with A and E nitrogen and R^3 and R^4 lower alkoxy.

38. Another preferred form of the invention has X = NH, n = 0, the aromatic ring phenyl optionally substituted, B & D carbon, with A and E nitrogen and R^3 and R^4 lower mono or dialkylamino.

39. Another preferred form of the invention has X = NH, n = 0, the aromatic ring phenyl optionally substituted, B & D carbon, with A and E nitrogen and R^3 or R^4 lower alkoxy, with the other lower mono or dialkylamino.

40. Another preferred form of the invention has X = NH, n = 0, the aromatic ring phenyl optionally substituted, B & D carbon, with A and E nitrogen and

R^3 and R^4 taken together are ethylenedioxy, 2,3-fused piperazine, 2,3-fused morpholine or 2,3-fused thiomorpholine.

Scheme 17 -
Route to Preferred Groups 41 [3,2-d] ring fusion

3,H-Thieno[3,2-d]pyrimid-4-one can be made by standard chemistry from commercially available ethyl 3-aminothiophene carboxylate and formamide. Conversion of the carbonyl to chloride by standard techniques followed by displacement with an appropriate amine gives the desired thieno[3,2-d]pyrimidines. If R^4 is not H, an appropriate electrophile, for example nitro for amine based or diazotization derived substituents, or Br for Stille coupled final products, can be introduced either at the stage shown or an earlier stage, and then be converted to R^4, by reduction and amination for example or by Stille coupling, or other methods known to those skilled in the art. [This technique follows also for all of the following preferred categories which have the possibility of substitution on R^3 or R^4, as they are all contain electron rich five membered rings which can be readily manipulated by electrophilic aromatic substitution.] (DMSO is dimethyl sulfoxide).

41. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, and either A and B taken together are a sulfur atom, with D & E carbon, or A & B are carbon with D and E taken together as a sulfur atom, with R^4 or R^3 H, lower alkyl, lower alkoxy, amino, or lower mono or dialkylamino.

Scheme 18 -
Route to Preferred Groups 41 [2,3-d] ring fusion

Thieno[2,3-d]pyrimid-4-one is built up by the Gewald synthesis from 2,5-dihydroxy-1,4-dithiane and ethyl cyanoacetate, followed by formamide cyclization. Conversion of the carbonyl to chloride by standard techniques followed by displacement with an appropriate amine gives the desired thieno[2,3-d]pyrimidines.

Scheme 19 -
Route to Preferred Groups 42 [3,2-d] ring fusion

The [3,2-d] ring fusion compounds are obtained from 3-bromofurfural as shown above in Scheme A. Displacement of the bromide by azide, followed by oxidation of the aldehyde sets up the basic aminofuroic acid needed to fuse on the pyrimidine ring. The annulation shown can be used, or by manipulating which acid derivative is actually used, one could use a variety of other ring annulations, and subsequent activations of the 4-position if required.

42. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, and either A and B taken together are an oxygen atom, with D & E carbon, or A & B are carbon with D and E taken together as an oxygen atom, with R^4 or R^3 H, lower alkyl, lower alkoxy, amino, or lower mono or dialkylamino.

aldehyde,which is treated with hydroxylamine under mild acidic conditions, and then basic conditions to complete the ring annulation giving 4-methylthioisoxazolo[5,4-d]pyrimidine, which on displacement with an appropriate amine gives the desired isoxazolo[5,4-d]pyrimidine derivatives as shown above.

47. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, and either A and B taken together are an oxygen atom with D nitrogen and E carbon, or A and B taken together are a carbon atom with D nitrogen and E oxygen, with $R^{3/6}$ H, lower alkyl, lower alkoxy, amino, or lower mono or dialkylamino.

Scheme 29 -
Route to Preferred Groups 47 [4,5-d] ring fusion

Reaction of 4,6-dichloro-5-nitropyrimidine with CuCN/NMP gives the 4-nitrile. Reduction of the nitro group to the corresponding amine is followed by diazotization and treatment with dilute sulfuric acid to give the corresponding 5-hydroxy compound. Reaction of this with Me_3Al/ NH_4Cl gives the amidine which is oxidatively cyclized to 7-amino-4-chloroisoxazolo[4,5-d]pyrimidine. Removal of the amino functionality by diazotization/hypophosphorus acid is followed by displacement of the 4-chlorine with an appropriate amine to give the desired isoxazolo[4,5-d]pyrimidine derivatives as shown above.

Scheme 30 -
Route to Preferred Groups 48 [5,4-d] ring fusion

Reaction of 6-chloro-4-methylthiopyrimidine with LDA followed by DMF gives the corresponding 5-aldehyde,which is treated sequentially with NaSH, NBS and ammonia to complete the ring annulation giving 4-methylthioisothiazolo[5,4-d]pyrimidine, which on displacement with an appropriate amine gives the desired isothiazolo[5,4-d]pyrimidine derivatives as shown above.

48. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, and either A and B taken together are a sulfur atom with D nitrogen and E carbon, or A and B taken together are a carbon atom with D nitrogen and E sulfur, with $R^{3/6}$ H, lower alkyl, lower alkoxy, amino, or lower mono or dialkylamino.

Scheme 31 -
Route to Preferred Groups 48 [4,5-d] ring fusion

Reaction of 4,6-dichloro-5-nitropyrimidine with CuCN/NMP gives the 4-nitrile. Reduction of the nitro group to the amine is followed by diazotization/thiation to give the corresponding 5-mercapto compound. Reaction of this with Me_3Al/ NH_4Cl gives the amidine which is oxidatively cyclized with NBS to 7-amino-4-chloroisothiazolo[4,5-d]pyrimidine. Removal of the amino functionality by diazotization/hypophosphorus acid is followed by displacement of the 4-chlorine with an appropriate

amine to give the desired isothiazolo4,3-d]pyrimidine derivatives as shown above.

Scheme 32 -
Route to Preferred Groups 49 [3,4-d] ring fusion

Reaction of 6-chloro-4-methylthiopyrimidine with LDA followed by DMF gives the corresponding 5-aldehyde, which is treated with hydrazine to do the ring annulation giving 4-methylthiopyrazolo[3,4-d]pyrimidine, which on displacement with an appropriate amine gives the desired pyrazolo[3,4-d]pyrimidine derivatives as shown above.

49. Another preferred form of the invention has X = NH, n = 0 or 1, in which case R^1 = H, the aromatic ring phenyl optionally substituted, and either A and B taken together are a nitrogen atom with D nitrogen and E carbon, or A and B taken together are a carbon atom with D and E nitrogen atoms, with $R^{3/6}$ H or lower alkyl if on nitrogen, or H, lower alkyl, lower alkoxy, amino, or lower mono or dialkylamino if on carbon.

Scheme 33 -
Route to Preferred Groups 49 [4,3-d] ring fusion

Nitration of pyrazole-3-carboxylic acid followed by reduction gives 4-aminopyrazole-3-carboxylic acid. This is cyclized to pyrazolo[4,3-d]pyrimid-4-one with formamidine HCl, and replacement of the carbonyl with halide by standard procedures, followed by displacement of the chloride by an appropriate amine yields the desired pyrazolo[4,3-d]pyrimidine, as shown above.

Most Preferred Forms of the Invention

1. A most preferred form of the invention is one where X = NH, x = 0, the aromatic ring is 3-bromophenyl, B, D & E are carbon, A is nitrogen, and R^4 is amino.

2. A most preferred form of the invention is one where X = NH, x = 0, the aromatic ring is 3-bromophenyl, B, D & E are carbon, A is nitrogen, and R^4 is methylamino.

3. A most preferred form of the invention is one where X = NH, x = 0, the aromatic ring is 3-bromophenyl, B, D & E are carbon, A is nitrogen, and R^4 is dimethylamino.

4. A most preferred form of the invention is one where X = NH, x = 0, the aromatic ring is 3-nitrophenyl, A, D & E are carbon, B is nitrogen, and R^4 is amino.

5. A most preferred form of the invention is one where X = NH, x = 0, the aromatic ring is 3-bromophenyl, A, D & E are carbon, B is nitrogen, and R^4 is amino.

6. A most preferred form of the invention is one where X = NH, x = 0, the aromatic ring is 4-bromophenyl, A, D & E are carbon, B is nitrogen, and R^4 is amino.

7. A most preferred form of the invention is one where X = NH, x = 0, the aromatic ring is 3-

trifluoromethylphenyl, A, D & E are carbon, B is nitrogen, and R^4 is amino.

8. A most preferred form of the invention is one where X = NH, x = 0, the aromatic ring is 3-bromophenyl, A, D & E are carbon, B is nitrogen, and R^4 is acetylamino.

9. A most preferred form of the invention is one where X = NH, x = 1, R^1 = H, the aromatic ring is phenyl, A, D & E are carbon, B is nitrogen.

10. A most preferred form of the invention is one where X = NH, x = 1, R^1 = H, the aromatic ring is phenyl, A, D & E are carbon, B is nitrogen, and R^4 is acetylamino.

11. A most preferred form of the invention is one where X = NH, x = 0, the aromatic ring is 3-bromophenyl, A, B & E are carbon, D is nitrogen, R^3 = Cl.

12. A most preferred form of the invention is one where X = NH, x = 0, the aromatic ring is 3-bromophenyl, A, D & E are carbon, D is nitrogen, and R^3 is methoxy.

13. A most preferred form of the invention is one where X = NH, x = 0, the aromatic ring is 3-bromophenyl, A, D & E are carbon, D is nitrogen, and R^3 is methylamino.

14. A most preferred form of the invention is one where X = NH, x = 0, the aromatic ring is 3-

bromophenyl, A, D & E are carbon, D is nitrogen, and R^3 is dimethylamino.

15. A most preferred form of the invention is one where X = NH, x = 0, the aromatic ring is 3-bromophenyl, D & E are carbon, and A and B taken together are S.

16. A most preferred form of the invention is one where X = NH, x = 1, R^1 =H, the aromatic ring is phenyl, D & E are carbon, and A and B taken together are S.

17. A most preferred form of the invention is one where X = NH, x = 0, the aromatic ring is 3-bromophenyl, A & B are carbon, and D and E taken together are S.

18. A most preferred form of the invention is one where X = NH, x = 0, the aromatic ring is 3-bromophenyl, B is carbon, and A, and D and E taken together, are nitrogen.

19. A most preferred form of the invention is one where X = NH, x = 0, the aromatic ring is 3-bromophenyl, A, B & E are carbon, D is nitrogen, and R^3 is N-piperinyl.

20. A most preferred form of the invention is one where X = NH, x = 0, the aromatic ring is 3-bromophenyl, A, D & E are carbon, B is nitrogen, and R^4 is fluoro.

21. A most preferred form of the invention is one where X = NH, x = 0, the aromatic ring is 3-hydroxyphenyl, A, D & E are carbon, B is nitrogen, and R^4 is amino.

22. A most preferred form of the invention is one where X = NH, x = 0, the aromatic ring is 3-bromophenyl, A, D & E are carbon, B is nitrogen, and R^4 is methylamino.

23. A most preferred form of the invention is one where X = NH, x = 0, the aromatic ring is 3-bromophenyl, A, D & E are carbon, B is nitrogen, and R^4 is dimethylamino.

24. A most preferred form of the invention is one where X = NMe, x = 0, the aromatic ring is 3-bromophenyl, A, D & E are carbon, B is nitrogen, and R^4 is methylamino.

25. A most preferred form of the invention is one where X = NH, x = 0, the aromatic ring is 3-bromophenyl, A, D & E are carbon, B is nitrogen, and R^4 is methoxy.

26. A most preferred form of the invention is one where X = NH, x = 0, the aromatic ring is 3-bromophenyl, A, B & D are carbon, E is nitrogen, and R^4 is fluoro.

Biology

These compounds are potent and selective inhibitors of the human EGF receptor tyrosine kinase,

and other members of the EGF receptor family, including the ERB-B2, ERB-B3 and ERB-B4 receptor kinases, and are useful for the treatment of proliferative diseases in mammals. These inhibitors prevent mitogenesis in cells where mitogenesis is driven by one or more of this family of receptor kinases. This can include normal cells, where it is desired to prevent mitogenesis, as exemplified by the cells transformed by overexpression or mutation of this kinase family as exemplified by poor prognosis breast cancer where overexpression of EGFR, ERB-B2 and ERB-B3 or mutation of ERB-B2 to the oncoprotein NEU is a major factor in cellular transformation. As the preferred compounds are not highly cytotoxic and do not show potent growth inhibitory properties, because of their high specificity toward inhibition of the EGFR kinase family, they should have a much cleaner toxicity profile than most anti-cancer and anti-proliferative drugs. Their very different mode of action to current anti-cancer drugs should allow for their use in multiple drug therapies, where synergism with available agents is anticipated.

Compounds of the invention have been shown to be very potent, reversible inhibitors of the EGF receptor tyrosine kinase, by binding with high affinity at the adenosine triphosphate (ATP) binding site of the kinase. These compounds exhibit potent IC_{50}s, varying from 10 micromolar to 5 picomolar, for the tyrosine kinase activity of the enzyme, based on an assay examining phosphorylation of a peptide derived from the phosphorylation site of the protein PLCgamma1, a known EGFR phosphorylation substrate. This data is shown in Table 1.

Biological Data

Materials and Methods

Purification of Epidermal Growth Factor Receptor Tyrosine Kinase - Human EGF receptor tyrosine kinase was isolated from A431 human epidermoid carcinoma cells which overexpress EGF receptor by the following methods. Cells were grown in roller bottles in 50% Delbuco's Modified Eagle and 50% HAM F-12 nutrient media (Gibco) containing 10% fetal calf serum. Approximately 10^9 cells were lysed in two volumes of buffer containing 20 mM 2-(4N-[2-hydroxyethyl]piperazin-1-yl)ethanesulfonic acid (hepes), pH 7.4, 5 mM ethylene glycol bis(2-aminoethyl ether) N,N,N',N'-tetraacetic acid, 1% Triton X-100, 10% glycerol, 0.1 mM sodium orthovanadate, 5 mM sodium fluoride, 4 mM pyrophosphate, 4 mM benzamide, 1 mM dithiothreitol, 80 µg/mL aprotinin, 40 µg/mL leupeptin and 1 mM phenylmethylsulfonyl fluoride. After centrifugation at 25,000 x g for 10 minutes, the supernatant was equilibrated for 2 h at 4°C with 10 mL of wheat germ agglutinin sepharose that was previously equilibrated with 50 mM Hepes, 10% glycerol, 0.1% Triton X-100 and 150 mM NaCl, pH 7.5, (equilibration buffer). Contaminating proteins were washed from the resin with 1 M NaCl in equilibration buffer, and the enzyme was eluted with 0.5 M N-acetyl-1-D-glucosamine in equilibration buffer, followed by 1 mM urea. The enzyme was eluted with 0.1 mg/ml EGF. The receptor appeared to be homogeneous as assessed by Coomassie blue stained polyacrylamide electrophoretic gels.

Determination of IC_{50} values - enzyme assays for IC_{50} determinations were performed in a total volume of 0.1 mL, containing 25 mM Hepes, pH 7.4, 5 mM $MgCl_2$, 2 mM $MnCl_2$, 50 μM sodium vanadate, 5-10 ng of EGF receptor tyrosine kinase, 200 μM of a substrate peptide, (Ac-Lys-His-Lys-Lys-Leu-Ala-Glu-Gly-Ser-Ala-Tyr^{472}-Glu-Glu-Val-NH_2, derived from the amino acid (Tyr^{472} has been shown to be one of four tyrosines in PLC (phospholipaseC)-gamma 1 that are phosphorylated by the EGF receptor tyrosine kinase [Wahl, M. I.; Nishibe, S.; Kim, J. W.; Kim, H.; Rhee, S. G.; Carpenter, G., J. Biol. Chem., (1990), 265, 3944-3948.], and peptides derived from the enzyme sequence surrounding this site are excellent substrates for the enzyme.),10 μM ATP containing 1 μCi of [^{32}P]ATP and incubated for ten minutes at room temperature. The reaction was terminated by the addition of 2 mL of 75 mM phosphoric acid and passed through a 2.5 cm phosphocellulose filter disc to bind the peptide. The filter was washed five times with 75 mM phosphoric acid and placed in a vial along with 5 mL of scintillation fluid (Ready gel Beckman).

Table 1

EGF Receptor Tyrosine Kinase Inhibition

Example #	IC_{50}
1	8 μM
2	3.6 μM
3	1.1 μM
4	225 nM
5	1.9 μM
6	7.6 nM
7	3.1 nM
8	9.6 nM
9	405 nM
10	6.1 μM
11	194 nM
12	13 nM
13	250 nM
14	70 nM
15	134 nM
16	3.7 μM
17	1.55 μM
18	173 nM
19	1.8 μM
20	4.9μM
21	1.25 μM
22	39 nM
23	840 nM
24	123 nM
25	377 nM
26	241 nM
27	10 nM

Example #	IC_{50}
28	94 nM
29	262 nM
30	10 μM
31	15 nM
32	4.7 μM
33	130 pM
34	91 pM
35	3.1 nM
36	29 nM
37	39 nM
38	71 nM
39	590 nM
40	578 nM
41	220 nM
42	226 nM
43	10 μM
44	10 μM
45	2.87 μM
46	1.42 μM
47	1.67 μM
48	1.0 μM
49	2.5 μM
50	10 μM
51	1.95 μM
52	8 μM
53	1.8 μM
54	100 nM
55	400 nM
56	110 nM
57	124 nM

Example #	IC_{50}
58	40 nM
59	2.6 nM
60	8 pM
61	6 pM
62	6.1 μM
63	6.1 μM
64	11 nM
65	5.1 μM
66	190 nM
67	6.1 μM
68	263 nM
69	7.0 μM
70	473 nM
71	11 nM
72	35 nM
73	36 nM
74	11.5 μM
75	55 nM
76	10 μM
77	39 nM
78	670 nM
79	6.7 nM

Cells

Swiss 3T3 mouse fibroblasts, A431 human epidermoid carcinoma cells, and MCF-7 (Michigan Cancer Foundation human mammary carcinoma cells), SK-BR-3 (human mammary carcinoma cells), MDA-MB-231 and MDA-MB-468 (human mammary carcinoma cells) breast

carcinomas were obtained from the American Type Culture Collection, Rockville, Maryland and maintained as monolayers in dMEM (Dulbecco's modified eagle medium)/F12, 50:50 (Gibco/BRL) containing 10% fetal bovine serum. To obtain conditioned medium, MDA-MB-231 cells were grown to confluency in an 850 cm^2 roller bottle and the medium replaced with 50 ml of serum-free medium. After 3 days the conditioned medium was removed, frozen down in aliquots and used as a heregulin source to stimulate erbB-2, 3, 4.

Antibodies

Monoclonal antibodies raised to the PDGF (platelet-desired growth factor) receptor or phosphotyrosine were from Upstate Biotechnology, Inc., Lake Placid, NY. Anti-$pp39^{Jun}$ (antibody to the transcription factor c-jun, which is a 39 kDalton phosphoprotein) and anti-EGF receptor antibodies were from Oncogene Science, Uniondale, NY.

Immunoprecipitation and Western Blot

Cells were grown to 100% confluency in 100 mm Petrie dishes (Corning). After the cells were treated for 5 minutes with either EGF (epidermal growth factor), PDGF, or bFGF (basic fibroblast growth factor) (20 ng/ml) or 1 ml of conditioned media from MDA-MB-231 cells, the media was removed and the monolayer scraped into 1 ml of ice cold lysis buffer (50 mM Hepes, pH 7.5, 150 mM NaCl, 10% glycerol, 1% triton X-100, 1 mM EDTA, 1 mM EGTA, 10 mM sodium pyrophosphate, 30 mM p-nitrophenyl phosphate, 1 mM orthovanadate, 50 mM sodium fluoride, 1 mM

phenylmethylsulfonylfluoride, 10 μg/ml of aprotinin, and 10 μg/ml of leupeptin). The lysate was transferred to a microfuge tube (small centrifuge that holds 1-2 ml plastic centrifuge tubes), allowed to sit on ice 15 minutes and centrifuged 5 minutes at 10,000 x g. The supernatant was transferred to a clean microfuge tube and 5 μg of antibody was added to designated samples. The tubes were rotated for 2 hours at 4° C after which 25 μl of protein A sepharose was added and then rotation continued for at least 2 more hours. The protein A separose was washed 5 times with 50 mM Hepes, pH 7.5, 150 mM NaCl, 10% glycerol and 0.02% sodium azide. The precipitates were resuspended with 30 μl of Laemlli buffer (Laemmli, NATURE, Vol. 727, pp. 680-685, 1970), heated to 100°C for 5 minutes and centrifuged to obtain the supernatant. Whole cell extracts were made by scraping cells grown in the wells of 6 well plates into 0.2 ml of boiling Laemmli buffer. The extract were transferred to a microfuge tube and heated to 100° C for 5 minutes. The entire supernatant from the immunoprecipitation or 35 μl of the whole cell extract was loaded onto a polyacrylamide gel (4-20%) and electrophoresis carried out by the method of Laemlli (Laemmli, 1970). Proteins in the gel were electrophoretically transferred to nitrocellulose and the membrane was washed once in 10 mM Tris buffer, pH 7.2, 150 mM NaCl, 0.01% Azide (TNA) and blocked overnight in TNA containing 5% bovine serum albumin and 1% ovalbumin (blocking buffer). The membrane was blotted for 2 hours with the primary antibody (1μg/ml in blocking buffer) and then washed 2 times sequentially in TNA, TNA containing 0.05% Tween-20 and 0.05% Nonidet P-40 (commercially available detergent) and TNA. The membranes were then incubated for 2 hours in blocking buffer containing 0.1 μCi/ml of [^{125}I] protein A and then washed again as above.

After the blots were dry they were loaded into a film cassette and exposed to X-AR X-ray film for 1-7 days. Protein A is a bacterial protein that specifically bonds certain IgG subtypes and is useful in binding to and isolating antibody-antigen complexes.

Northern Blots

Total cellular RNA was isolated from untreated control or treated Swiss 3T3 cells using RNAzol-B (trademark of Tel Test Inc. for a kit used to isolate RNA from tissues) and adhered to the protocol described by the manufacturer. Forty to fifty μg of RNA was loaded onto a 1% agarose gel and electrophoresis carried out for 3-4 hours at 65 volts. The RNA in the gel was transferred by capillary action to a nylon membrane (Hybond-N, Amersham). The 40 mer c-jun probe was end labeled with [^{32}P]ATP using T4 nucleotide kinase (Promega) and purified on a G25 sephadex column according to the procedure recommended by the supplier, Oncogene Science. Hybridization was performed overnight at 65°C (c-jun is an immediate early transcription factor; it is one of the components of AP-1 while FOS is the second component of AP-1.

Growth Factor-Mediated Mitogenesis

Swiss 3T3 fibroblasts were grown to 90 - 100% confluency in 24- well plates (1.7 x 1.6 cm, flat bottom) and growth arrested in serum-free media for 18 hours. Drug was added to specifi d wells 2 hours prior to growth factors and then the c lls were exposed to either 20 ng/ml EGF, PDGF or bFGF or 10% serum for 24

hours. Two μCi of [methyl-^{3}H]thymidine was added to each well and incubated for 2 hours at 37° C. The cells were trypsinized and injected into 2 ml of ice cold 15% trichloroacetic acid (TCA). The resulting precipitate was collected on glassfiber filters, washed five times with 2-ml aliquots of ice-cold 15% TCA, dried and placed in scintillation vials along with 10 ml Ready gel (Beckman, Irvine, CA). Radioactivity was determined in a Beckman LS 6800 scintillation counter.

Growth Inhibition Assay

Cells (2 x 10^4) were seeded in 24-well plates (1.7 x 1.6 cm, flat bottom) in two mls of medium with or without various concentrations of drug. Plates were incubated for 3 days at 37° in a humidified atmosphere containing 5% CO_2 in air. Cell growth was determined by cell count with a Coulter Model AM electronic cell counter (Coulter Electronics, Inc., Hialeah, FL).

INHIBITION OF EGF-INDUCED AUTOPHOSPHORYLATION IN A431 EPIDERMOID CARCINOMA CELLS AND CONDITIONED MEDIA-INDUCED AUTOPHOSPHORYLATION IN SK-BR-3 BREAST TUMOR CELLS BY COMPOUNDS OF THE CURRENT INVENTION

Example #	EGFR IC_{50} nM	A431 IC_{50} nM	SKBR-3 IC_{50} nM
4	225	>1000	>10,000
6	7.6	53	2660
7	3.1	20	100
8	9.6	32	71
22	39	252	~1500
27	10	110	~800
59	2.6	12	<10

Example #	EGFR IC_{50} nM	A431 IC_{50} nM	SKBR-3 IC_{50} nM
60	0.008	13	<10
61	0.006	21	39
70	11	124	<10
74	55	>1000	>1000

ANTIPROLIFERATIVE PROPERTIES OF TYROSINE KINASE INHIBITORS IC_{50} (nM)

	Ex 60	Ex 61
B104-1-1	2100	1000
SK-BR-3	600	900
MDA-468	3000	12000

B104-1-1 - NIH-3T3 fibroblasts transfected by the neu oncogene, Stem et al., *Science*, 234, pp. 321-324 (1987)

SK-BR-3 - Human breast carcinoma overexpressing erbB-2 and erbB-3

MDA-468 - Human breast carcinoma overexpressing the EGF receptor

The above gels, developed as detailed in the experimental section, demonstrate the efficacy of compounds of the current invention at blocking certain EGF-stimulated mitogenic signalling events in whole cells. The numbers to the left of the gels indicated the positions of molecular weight standards in kiloDaltons. The lane labelled control shows the degree of expression of the growth-related signal in the absence of EGF stimulation, whereas the lane labelled EGF (or PDGF or b-FGF) shows the magnitude of the growth factor-stimulated signal. The other lanes show the effect of the stated quantities of the named drug on the growth factor-stimulated activity being

measured, demonstrating that the compounds of the present invention have potent effects in whole cells, consistent with their ability to inhibit the tyrosine kinase activity of the EGF receptor.

Gel of Example 40 (Fig. 7) detects mRNA for c-jun by hybridization with a specific radiolabelled RNA probe for c-jun. The gel demonstrates that the growth factors EFG, PDGF and b-FGF stimulate c-jun production in Swiss 3T3 cells, and that compound 40 blocks this production for EGF-stimulated cells, but not for PDGF or b-FGF stimulated cells.

Effect of Example 40 on Growth Factor Mediated Expression of $p39^{c\text{-}jun}$

This gel shows the amount of c-jun induced in Swiss 3T3 cells by the growth factor EGF, PDGF and b-FGF, quantitating with an anti-c-jun-specific monoclonal antibody. It demonstrates the ability of Example 40 to block c-jun expression in Swiss 3T3 when stimulated by EGF, but not when stimulated by PDGF or b-FGF.

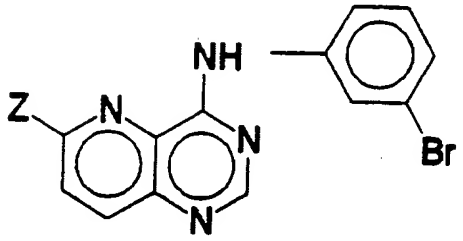
It is to be appreciated that the compounds described herein can be used in combination with other components to enhance their activity. Such additional components are anti-neoplastic materials as, doxorubicin, taxol, cis platin, and the like.

It has been found that the compounds described herein may inhibit both the erb-B2 and erb-B4 receptors and therefore have significantly increased clinical activity advantageously in

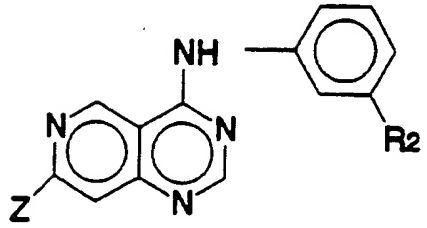
combination with the aforementioned anti-neoplastic agents.

See also the results shown in Figures 1 - 17.

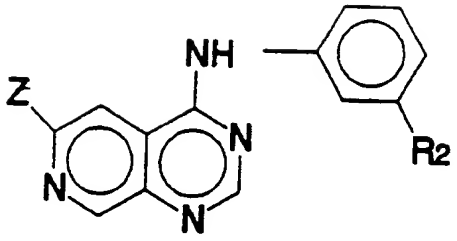
Some preferred structures are as follows:



Ex.#	Z
4	- fluorine
6	- NH_2
7	- $NHCH_3$
8	- $N(CH_3)_2$



Ex #	Z	R_2
22	NH_2	$-NO_2$
27	NH_2	Br



Ex #	Z	R_2
59	$-OCH_3$	Br
60	$-NH\ CH_3$	Br
61	$-N(CH_3)_2$	Br

Chemical Experimental

Listed below are preferred embodiments wherein all temperatures are in degrees Centigrade and all parts are parts by weight unless otherwise indicated.

Example 1

4-Anilinopyrido[3,2-d]pyrimidine mesylate

3H-Pyrido[3,2-d]pyrimidin-4-one. A solution of 6-chloro-3-nitropicolinamide (2.00 g, 9.91 mmol) in EtOAc/MeOH (1:1, 100 mL) is hydrogenated over 5% Pd-C (0.40 g) at 60 psi for 6 days, with additions of fresh catalyst after 2 and 4 days. After removal of the catalyst by filtration the solution is concentrated to dryness, to give 3-aminopicolinamide as an orange oil, which is used directly in the next step. The crude product is stirred under reflux with triethyl orthoformate (50 mL) for 42 h, during which time a tan precipitate forms. After cooling, the solid is filtered off, washed well with petroleum ether, and dried under vacuum to give 3H-pyrido[3,2-d]pyrimidin-4-one (1.27g, 87%), mp 343-345 °C [Price, C.C. and Curtin, D.Y. J. Amer. Chem. Soc. 68, 914, 1946 report mp 346-347 °C].

4-Chloropyrido[3,2-d]pyrimidine. A suspension of the above pyrimidinone (1.00 g, 6.80 mmol) in $POCl_3$ (30 mL) is heated under reflux for 4 h, and then concentrated to dryness under reduced pressure. The residue is partitioned between CH_2Cl_2 and saturated $NaHCO_3$ solution, and the organic layer worked up to give 4-chloropyrido[3,2-d]pyrimidine (0.97 g, 86%) as a tan solid, mp 335 °C (dec), which is used without further characterisation.

4-Anilinopyrido[3,2-d]pyrimidine mesylate. A solution of 4-chloropyrido[3,2-d]pyrimidine (84 mg, 0.5 mmol), aniline (56 mg, 0.6 mmol) and triethylamine (62 mg, 0.6 mmol) in EtOH (2 mL) are refluxed under N_2

with stirring for 2 h. The crude reaction mixture is purified on a preparative tlc plate (silica), eluting once with 3% MeOH in $CHCl_3$. The major band is extracted, and evaporated to dryness under reduced pressure, and the residual solid is dissolved in acetone, (5 mL), filtered, and methanesulfonic acid (32 μL, 0.5 mmol) is added slowly with swirling. The precipitate is collected by suction filtration, rinsed with acetone and dried in a vacuum oven to give 4-anilinopyrido[3,2-d]pyrimidine mesylate (91 mg, 57%) as dull yellow needles. ^1H NMR (DMSO) δ 11.75 (1H, slbrs), 9.11 (1H, dd, J = 1.5, 4.3 Hz), 8.97 (1H, s), 8.32 (1H, dd, J = 1.5, 8.4 Hz), 8.12 (1H, dd, J = 4.3, 8.5 Hz), 7.88 (2H, d, J = 8.2 Hz), 7.49 (2H, t, J = 8.0 Hz), 7.32 (1H, t, J = 7.0 Hz), 2.34 (3H, s).

Example 2

4-Benzylaminopyrido[3,2-d]pyrimidine

A solution of freshly prepared 4-chloropyrido[3,2-d]pyrimidine (0.10 g, 0.60 mmol) (prepared as described in the previous experimental) and benzylamine (0.13 mL, 1.20 mmol) in propan-2-ol (15 mL) containing a trace of conc. HCl is warmed at 50 °C for 30 min, and then concentrated to dryness. The residue is partitioned between water and EtOAc, and the organic layer worked up and chromatographed on silica gel. EtOAc elutes foreruns, while MeOH/EtOAc (1:9) elutes 4-(benzylamino)pyrido[3,2-d]pyrimidine (0.11 g, 77%). ^1H NMR ($CDCl_3$) δ 8.67 (1H, s), 6.50 (1H, dd, J = 4.3, 1.5 Hz), 8.10 (1H, dd, J = 8.5, 1.5 Hz), 7.63 (1H, dd, J = 8.8, 4.3 Hz), 7.55 (1H, brs), 7.41-7.29 (5H, m), 4.86 (2H, d, J = 5.9 Hz).

Example 3

4-(3-Bromoanilino)pyrido[3,2-d]pyrimidine

Reaction of 4-chloropyrido[3,2-d]pyrimidine (prepared as described in a previous experimental) with 3-bromoaniline in propan-2-ol containing a trace of conc. HCl at 50 °C for 30 min, followed by chromatography of the product on silica gel, gives 4-(3-bromophenyl)aminopyrido[3,2-d]pyrimidine (87% yield). ^{1}H NMR ($CDCl_3$) δ 9.19 (1H, brs), 8.83 (1H, s), 8.80 (1H, dd, J = 4.3, 1.5 Hz), 8.29 (1H, brs), 8.19 (1H, dd, J = 8.5, 1.5 Hz), 7.83 (1H, m), 7.76 (1H, dd, J = 8.5, 4.3 Hz), 7.29-7.27 (2H, m).

Example 4

4-(3-Bromoanilino)-6-fluoropyrido[3,2-d]pyrimidine

2-cyano-6-fluoro-3-nitropyridine. A mixture of 6-chloro-2-cyano-3-nitropyridine [Colbry, N. L.; Elslager, E. F. ; Werbel, L. M.; *J. Het. Chem.*, **1984**, *21*, 1521-1525] (10.0 g, 0.054 mol) and KF (9.48 g, 0.163 mol) in MeCN (200 mL) is heated under reflux with stirring for 18 h, then poured into water and extracted with EtOAc. The extract is washed with water and worked up, and the residue is chromatographed on silica gel, eluting with EtOAc/petroleum ether (3:7), to give after removal of the solvent under reduced pressure 2-cyano-6-fluoro-3-nitropyridine (7.2 g, 79%). ^{1}H NMR ($CDCl_3$) δ 8.79 (1H, dd, J = 9.0, 6.0 Hz), 7.48 (1H, dd, J = 9.0, 3.0 Hz).

6-Fluoro-3-nitropyridine-2-carboxamide. A solution of 2-cyano-6-fluoro-3-nitropyridine (1.40 g, 8.39 mmol) in 90% H_2SO_4 (30 mL) is warmed at 70 °C for

90 min, then cooled, poured onto ice and basified with conc. ammonia. Extraction with EtOAc and workup gives 6-fluoro-3-nitropyridine-2-carboxamide (0.94 g, 61%). ^{1}H NMR ($CDCl_3$) δ 8.70 (1H, dd, J = 8.9, 6.5 Hz), 8.30, 8.03 (1H, 1H, brs), 7.62 (1H, dd, J = 8.9, 2.9 Hz).

6-Fluoro-3H-pyrido[3,2-d]pyrimid-4-one. A solution of 6-fluoro-3-nitropyridine-2-carboxamide (1.50 g, 8.10 mmol) in EtOAc (80 mL) is hydrogenated over 5% Pd-C (0.30 g) at 60 psi for 2 h. After removal of the catalyst by filtration, the solvent is removed under reduced pressure, to give a residue of crude 3-amino-6-fluoropyridine-2-carboxamide which is used directly in the next step. Triethyl orthoformate (60 mL) is added and the mixture is then heated under reflux with vigorous stirring for 18 h. The cooled mixture is diluted with an equal volume of petroleum ether, and the resulting precipitate collected by filtration and is washed well with petroleum ether to give 6-fluoro-3H-pyrido[3,2-d]pyrimid-4-one (1.26 g, 84%). ^{1}H NMR (DMSO) δ 12.72 (1H, brs), 8.31 (1H, dd, J = 8.6, 7.7 Hz), 8.20 (1H, s), 7.66 (1H, dd, J = 8.6, 3.0 Hz).

4-(3-Bromoanilino)-6-fluoropyrido[3,2-d]pyrimidine. A suspension of 6-fluoro-3H-pyrido[3,2-d]pyrimid-4-one (0.20 g, 1.21 mmol) in $POCl_3$ (30 mL) is heated under reflux with stirring until homogeneous (2 h), and then for a further 1 h. Excess $POCl_3$ is removed under reduced pressure, and the residue is partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. Workup of the organic portion gives crude 4-chloro-6-fluoropyrido[3,2-d]pyrimidine (100%) as an unstable white solid which is used directly in the next step.

A solution of 4-chloro-6-fluoropyrido[3,2-d]pyrimidine (0.20 g, 1.1 mmol) and 3-bromoaniline (0.12 mL, 2.18 mmol) in propan-2-ol (20 mL) containing conc. HCl (1 drop) is heated under reflux for 15 min, then cooled, poured into water and extracted with EtOAc. The extract is worked up, and the residue chromatographed on silica gel, eluting with EtOAc/petroleum ether (1:2)to give after removal of the solvent under reduced pressure 4-(3-bromoanilino)-6-fluoropyrido[3,2-d]pyrimidine (0.18 g, 52%). ^{1}H NMR ($CDCl_3$) δ 8.82 (1H, s), 8.65 (1H, brs), 8.31 (1H, t, J = 7.4 Hz), 8.27 (1H, brs), 7.77 (1H, m) 7.41 (1H, dd, J = 8.9, 2.2 Hz), 7.29 (2H, brs).

Example 5

4-(3-Bromoanilino)-6-chloropyrido[3,2-d]pyrimidine

6-chloro-3-nitropicolinamide. A solution of 6-chloro-3-nitropicolinonitrile (1.00 g, 5.45 mmol) in 90% H_2SO_4 (15 mL) is warmed at 70 °C for 3.5 h, and then poured into ice-water. The mixture is extracted four times with EtOAc and the combined extracts worked up to give 6-chloro-3-nitropicolinamide (0.80 g, 73%). ^{1}H NMR (DMSO) δ 8.55 (1H, d, J = 8.5 Hz), 8.31, 8.04 (1H, 1H, 2 brs), 7.93 (1H, d, J = 8.5 Hz).

6-Chloro-3H-pyrido[3,2-d]pyrimidin-4-one. A solution of 6-chloro-3-nitropicolinamide (0.30 g, 1.49 mmol) in EtOAc (30 mL) is hydrogenated at 60 psi over 5% Pd-C (0.10 g) for 20 min. After removal of the catalyst by filtration the solution is concentrated to dryness to give 3-amino-6-chloropicolinamide as a yellow oil, which is used directly in the next step. It is dissolved in triethylorthoformate (30 mL) and

the mixture is heated under reflux for 18 h. Petroleum ether (30 mL) is added to the cooled solution, and the resulting precipitate of crude 6-chloro-3H-pyrido[3,2-d]pyrimidin-4-one (0.27 g, 99%) is filtered off and dried in a vacuum oven.

4-(3-Bromoanilino)-6-chloropyrido[3,2-d]pyrimidine. A suspension of the above quinazolone (0.20 g, 1.10 mmol) in $POCl_3$ (30 mL) is heated under reflux for 3 h, and then concentrated to dryness under reduced pressure. The residue is partitioned between CH_2Cl_2 and saturated $NaHCO_3$ solution, and the organic portion is worked up to give 4,6-dichloropyrido[3,2-d]pyrimidine (0.16 g, 73%) as a tan solid, which is used directly in the next step. A solution of the crude dichloropyridopyrimidine (0.16 g, 0.80 mmol) and 3-bromoaniline (0.17 mL, 1.60 mmol) in propan-2-ol (25 mL) containing a trace of conc. HCl is warmed at 50 °C for 30 min. The cooled mixture is poured into saturated $NaHCO_3$ and extracted with EtOAc, and the extract is worked up and chromatographed on silica gel. Elution with EtOAc/petroleum ether (1:4) gives 3-bromoaniline, while EtOAc/petroleum ether (1:1) elutes 4-(3-bromoanilino)-6-chloropyrido[3,2-d]pyrimidine (0.17 g, 63%). ^1H NMR ($CDCl_3$) δ 8.90 (1H, brs,) 8.84 (1H, s), 8.30 (1H, dd, J = 2.1, 2.0 Hz) 8.17 (1H, d, J = 8.8 Hz), 7.82-7.78 (1H, m) 7.73 (1H, d, J = 8.8 Hz), 7.32-7.29, (2H, m).

Example 6

4-(3-Bromoanilino)-6-aminopyrido[3,2-d]pyrimidine

Reaction of 4-(3-bromoanilino)-6-fluoropyrido[3,2-d]pyrimidine (0.12 g, 0.38

mmol) (described in a previous experimental) with a saturated solution of ammonia in ethanol in a pressure vessel at 100 °C for 18 h gives 6-amino-4-(3-bromoanilino)pyrido[3,2-d]pyrimidine, (87 mg, 72%). ^{1}H NMR ($CDCl_3$) δ 8.76 (1H, brs), 8.64 (1H, s), 8.23 (1H, brs), 7.93 (1H, d, J = 9.0 Hz), 7.81 (1H, dt, J_d = 7.7 Hz, J_t =1.8 Hz), 7.28-7.22 (2H, m), 7.00 (1H, d, J = 9.0 Hz), 4.90 (2H, brs).

Example 7

4-(3-Bromoanilino)-6-methylaminopyrido[3,2-d]pyrimidine

Reaction of 4-(3-bromoanilino)-6-fluoropyrido[3,2-d]pyrimidine (50 mg, 0.16 mmol) (described in a previous experimental) with methylamine hydrochloride (32 mg, 0.47 mmol) and triethylamine (70 μL, 0.55 mmol) in ethanol (10 mL) in a pressure vessel at 100 °C for 18 h gives 6-methylamino-4-(3-bromoanilino)pyrido[3,2-d]pyrimidine (43 mg, 81%). ^{1}H NMR ($CDCl_3$) δ 8.81 (1H, brs), 8.61 (1H, s), 8.19 (1H, t, J = 1.8 Hz), 7.86 (1H, d, J = 9.1 Hz,), 7.83 (1H, dt, J_d = 7.7 Hz, J_t =1.8 Hz), 7.28-7.21 (2H, m), 6.92 (1H, d, J = 9.1 Hz), 4.97 (1H, q, J = 5.0 Hz), 3.13 (3H, d, J = 5.0 Hz).

Example 8

4-(3-Bromoanilino)-6-dimethylaminopyrido[3,2-d]pyrimidine.

A mixture of 4-(3-bromoanilino)-6-fluoropyrido[3,2-d]pyrimidine (0.15 g, 0.47 mmol) (described in a previous experimental), dimethylamine hydrochloride (0.11 g, 1.41 mmol) and triethylamine

(0.23 mL, 1.64 mmol) in EtOH (15 mL) is heated in a pressure vessel at 100 °C for 18 h. The solvent is removed under reduced pressure, and the residue is partitioned between EtOAc and water. The organic portion is worked up, and the residue chromatographed on silica gel. Elution with EtOAc/petroleum ether (1:1) gives foreruns, while EtOAc elutes off 4-(3-bromoanilino)-6-dimethylaminopyrido[3,2-d]pyrimidine (0.14 g, 86%). ^{1}H NMR ($CDCl_3$) δ 8.72 (1H, brs), 8.56 (1H, s), 8.17 (1H, t, J = 1.9 Hz), 7.85 (1H, d, J = 9.3 Hz), 7.77 (1H, dt, J_d = 7.5 Hz, J_t =1.9 Hz), 7.27-7.18 (2H, m), 7.08 (1H, d, J = 9.3 Hz), 3.21 (6H, s).

Example 9

4-(3-Bromoanilino)-6-methoxypyrido[3,2-d]pyrimidine

4-(3-Bromoanilino)-6-fluoropyrido[3,2-d]pyrimidine (described in a previous experimental) (0.11 g, 0.34 mmol) is added to a solution of NaOMe (prepared by the addition of Na metal (31 mg, 1.38 mmol) to dry MeOH (15 mL). After heating in a pressure vessel at 90 °C for 3 h, the solution is concentrated to dryness and the residue is partitioned between EtOAc and water. Workup of the organic portion gives 4-(3-bromophenyl)amino-6-methoxypyrido[3,2-d]pyrimidine (92 mg, 82%). ^{1}H NMR ($CDCl_3$) δ 8.73 (1H, s), 8.66 (1H, brs), 8.18 (1H, m), 8.05 (1H, d, J = 8.9 Hz), 7.83-7.80 (1H, m), 7.30-7.24 (2H, m), 7.23 (1H, d, J = 8.9 Hz), 4.12 (3H, s).

Example 10

4-Anilinopyrido[4,3-d]pyrimidine

4-(N-t-Butoxycarbonylamino)pyridine. To a mixture of 4-aminopyridine (2 g, 21.24 mmol), potassium hydroxide (3.57 g, 63.72 mmol), water (10 mL), and 2-methyl-2-propanol (4 mL) on ice is added di-t-butyl-dicarbonate (6.95 g, 31.87 mmol). The resulting biphasic solution is stirred at 25°C for 1 week, then water (20 mL) is added. The solution is extracted with 1X CH_2Cl_2 and 2X EtOAc. The organic layer is dried ($MgSO_4$) and concentrated under reduced pressure to give 4-(N-t-butoxycarbonylamino)pyridine (4.08 g, 99%). ^1H NMR (DMSO) δ 9.84 (1H, s), 8.35 (2H, d, J = 6 Hz), 7.44 (2H, d, J = 7 Hz), 1.49 (9H, s).

4-(N-t-Butoxycarbonylamino)nicotinic acid. n-Butyl lithium (2.18 M, 24 mL, 52.51 mmol) is added slowly to a solution of 4-(N-t-butoxycarbonylamino)-pyridine (4.08 g, 21 mmol) in THF (50 mL, stirred under N_2 at -78 °C. The solution is allowed to warm to 0°C, stirred for 3 h, then cooled again to -78 °C and poured into ether (100 mL) containing dry ice. The solution is warmed to room temperature with constant stirring. Water is added and the mixture is neutralized with acetic acid. The resulting solid is collected by vacuum filtration and dried in a vacuum oven to give 4-(N-t-butoxycarbonylamino)nicotinic acid (2.72 g, 54%) as a brown solid. ^1H NMR (DMSO) δ 11.75 (1H, brs), 8.95 (1H, s), 8.50 (1H, d, J = 6.0 Hz), 8.20 (1H, d, J = 6.0 Hz), 1.49 (9H, s).

4-Amino nicotinic acid. A mixture of 4-(N-t-butoxycarbonylamino)nicotinic acid (2.72 g, 11.4

mmol), TFA (10 mL), and CH_2Cl_2 (20 mL) is stirred at room temperature for 12 h. The volatiles are removed under reduced pressure, and the resulting crude 4-amino nicotinic acid is used directly in the next reaction.

3H-Pyrido[4,3-d]pyrimidin-4-one. Crude 4-amino nicotinic acid (2.72 g, 11.4 mmol) in formamide (20 mL) is heated to 170°C for 12 h. The volatiles are distilled out under reduced pressure (0.8 mmHg). The residual solid is then purified on a medium pressure silica gel column, eluting with 10% MeOH in $CHCl_3$ to give 3H-pyrido[4,3-d]pyrimidin-4-one (780 mg, 47%) as a whitish yellow solid. 1H NMR (DMSO) δ 12.64 (1H, brs), 9.28 (1H, s), 8.83 (1H, d, J = 5.5 Hz), 8.30 (1H, s), 7.58 (1H, d, J = 5.8 Hz).

3H-Pyrido[4,3-d]pyrimidin-4-thione. Phosphorous pentasulfide (2.59 g, 5.83 mmol) is added to a solution of 3H-pyrido[4,3-d]pyrimidin-4-one (780 mg, 5.3 mmol) in pyridine (5 mL). The mixture is refluxed for 5 h. On cooling a precipitate forms and the supernatent is decanted off. The solid is suspended in water (20 mL) and then filtered to yield 3H-pyrido[4,3-d]pyrimidin-4-thione (676 mg, 78%) as a black solid. ^{1}H NMR (DMSO) δ 14.53 (1H, brs), 9.65 (1H, s), 8.84 (1H, d, J = 7.0 Hz), 8.32 (1H, s), 7.64 (1H, d, J = 8.0 Hz).

4-Methylthiopyrido[4,3-d]pyrimidine. A mixture of 3H-pyrido[4,3-d]pyrimidin-4-thione (676 mg, 4.14 mmol), triethylamine (1.4 mL, 10.31 mmol), DMSO (4 mL), and iodomethane (0.48 mL, 7.72 mmol) is stirred for 12 h under N_2 at 25°C. The mixture is

poured onto water and extracted with EtOAc. The organic extracts are dried ($MgSO_4$), and the solvent is removed under reduced pressure to yield 4-methylthiopyrido[4,3-d]pyrimidine (1.15 g, quant.) as a brown solid. ^1H NMR (DMSO) δ 9.52 (1H, s), 9.16 (1H, s), 8.95 (1H, d, J = 6 Hz), 7.86 (1H, d, J = 8 Hz), 2.75 (1H, s).

4-Anilinopyrido[4,3-d]pyrimidine. A mixture of 4-methylthiopyrido[4,3-d]pyrimidine (174 mg, 0.97 mmol), and aniline (186.2 mg, 1.99 mmol) in EtOH (2 mL) is refluxed under N_2 for 12 h. Cooling to 0°C forms a solid which is filtered to yield 4-anilinopyrido-[4,3-d]pyrimidine (34.5 mg, 16%). ^1H NMR (DMSO) δ 10.29 (1H, brs), 9.86 (1H, s), 8.82 (1H, d, J = 5.8 Hz), 8.72 (1H, s), 7.85 (2H, d, J = 7.5 Hz), 7.66 (1H, d, J = 5.5 Hz), 7.45 (2H, t, J = 8.0 Hz), 7.23 (1H, t, J = 7.3 Hz).

Example 11

4-(3-Bromoanilino)pyrido[4,3-d]pyrimidine

A mixture of 4-methylthiopyrido[4,3-d]pyrimidine (171 mg, 0.96 mmol), (see previous experimental) and 3-bromoaniline (1 mL) is heated to 100°C for 2 h. A solid precipitates on cooling and is collected by vacuum filtration and then recrystallized from EtOH to yield 4-(3-bromoanilino)pyrido[4,3-d]pyrimidine (30 mg, 10%). ^1H NMR (DMSO) δ 10.33 (1H, s), 9.86 (1H, s), 8.84 (1H, d, J = 5.8 Hz), 8.79 (1H, s), 8.22 (1H, s), 7.89 (1H, d, J = 7.2 Hz), 7.69 (1H, d, J = 5.8 Hz), 7.40 (2H, dt, J_d = 8.0 Hz, J_t = 1.5 Hz).

Example 12

4-(3-Bromoanilino)-7-fluoropyrido[4,3-d]pyrimidine

3-Cyano-4,6-diaminopyridine. Crude 2-bromo-3-cyano-4,6-diaminopyridine [W.J.Middleton, US Patent 2,790,806 (April 30, 1957), Du Pont; Chem. Abst. 51:P14829 (1957), see also next experimental] (15.1 g, 0.071 mole) is hydrogenated in THF/MeOH (200 mL, 2:1) containing KOAc (7.0 g, 0.071 mole) and 5% Pd/C (4 g) at 55 p.s.i. and 20 °C for 7 days. Filtration over celite, washing with THF/MeOH and removal of the solvent gives a solid, which is dissolved in dilute HCl and water. Adjustment of the solution pH to 10 (conc. NaOH) and cooling gives 3-cyano-4,6-diaminopyridine (6.58 g, 69%) as a yellow solid, mp 197-198 °C [Metzger, R.; Oberdorfer, J.; Schwager, C.; Thielecke, W.; Boldt, P. Liebigs Ann. Chem. 1980, 946-953 record mp (benzene) 205 °C]. Extraction of the remaining liquor with EtOAc (4 x 200 mL) gives further product (2.12 g, 22%). ^{1}H NMR (DMSO) δ 7.91 (1H, s), 6.26, 6.24 (2H, 2H, brs), 5.63 (1H, s).

4,6-Diamino-3-pyridylcarboxamide. 3-Cyano-4,6-diaminopyridine (4.30 g, 0.032 mole) is added to 90% H_2SO_4 (25 mL), then stirred at 60-70 °C for 3 h. The resulting solution is added to cold conc. NaOH (40%) to give a mixture of 4,6-diamino-3-pyridylcarboxamide and inorganic salts. An analytically pure sample is obtained by chromatography on alumina (10-50% MeOH/$CHCl_3$) to give a pale yellow solid. ^{1}H NMR (DMSO) δ 8.15 (1H, s), 6.91 (2H, brs), 7.7-6.3 (2H, brm), 5.78 (2H, brs), 5.56 (1H, s).

7-Amino-4-oxo-3*H*-pyrido[4,3-d]pyrimidine. Crude 4,6-diamino-3-pyridylcarboxamide (9.2 g) is heated in purified $(EtO)_3CH$ (distilled from Na, 60 mL) at 170 °C for 1.5 d. After removing the solvent, the residue is dissolved in hot 2 M NaOH, filtered, neutralized (conc. HCl) and cooled to give 7-amino-4-oxo-3*H*-pyrido[4,3-d]pyrimidine (3.57 g, 69% from the nitrile) as a light brown solid ^1H NMR (DMSO) δ 11.79 (1H, brs), 8.74 (1H, s), 7.97 (1H, s), 6.76 (2H, brs), 6.38 (1H, s).

7-Fluoro-4-oxo-3*H*-pyrido[4,3-d]pyrimidine. A solution of 7-amino-4-oxo-3*H*-pyrido[4,3-d]pyrimidine (1.00 g, 6.17 mmol) in 60% HBF_4 (25 mL) at 0 °C is treated with solid $NaNO_2$ (0.85 g, 12.3 mmol, added in portions over 2 h), and is then stirred at 0 °C for a further 1 h and at 20 °C for 30 min. The resulting mixture is ice-cooled, neutralized with saturated aqueous Na_2CO_3, and extracted with EtOAc (4 x 100 mL). The extract is washed with water, then filtered through silica gel[3] (EtOAc) to give 7-fluoro-4-oxo-3*H*-pyrido[4,3-d]pyrimidine (0.48 g, 47%) as a cream solid. ^1H NMR (DMSO) δ 12.69 (1H, brs), 9.01 (1H, s), 8.31 (1H, s), 7.34 (1H, s)

4-(3-Bromoanilino)-7-fluoropyrido[4,3-d]pyrimidine. A suspension of 7-fluoro-4-oxo-3*H*-pyrido[4,3-d]pyrimidine (0.23 g, 1.39 mmol) in $POCl_3$ (10 mL) is stirred under reflux for 3.5 h, and is then concentrated under vacuum. The resulting oil is ice-cooled, diluted with CH_2Cl_2 (100 mL), saturated aqueous Na_2CO_3 (40 mL) and ice, and stirred at 20 °C for 2 h. The CH_2Cl_2 extract is separated and the aqueous portion further extracted with CH_2Cl_2 (2 x 100 mL), and then

the combined extracts are dried (Na_2SO_4) and filtered to give crude 4-chloro-7-fluoropyrido[4,3-d]pyrimidine. 3-Bromoaniline (1.26 g, 7.35 mmole), 3-bromoaniline hydrochloride (20 mg) and dry isopropanol (5 mL) are added, then the resulting solution is concentrated under vacuum to remove the CH_2Cl_2 and stirred at 20 °C for 1 h. Upon addition of dilute $NaHCO_3$ and water, the product crystallises. Filtration, washing with water and CH_2Cl_2, gives pure 4-(3-bromoanilino)-7-fluoropyrido[4,3-d]pyrimidine (297 mg, 67 %) as a cream solid. ^1H NMR (DMSO) δ 10.38 (1H, brs), 9.59 (1H, s), 8.72 (1H, s), 8.17 (1H, s), 7.85 (1H, m), 7.38 (3H, m).

Example 13

7-Amino-4-anilinopyrido[4,3-d]pyrimidine

4,6-Diamino-2-bromo-3-cyanopyridine. HBr is bubbled for 2 h into a mixture of malononitrile (16.3 g, 0.247 mol) and toluene (400 mL) at 0°C. A light yellow precipitate forms. The reaction mixture is then heated at 100°C for 2 h, with much gas evolution. After cooling to room temperature, the yellow solid is isolated via suction filtration, washed with toluene and air dried. The solid (25.96 g) is mixed with water (500 mL), and the pH of the suspension is adjusted to 9 ~ 10 with NH_4OH (conc. ~ 15 mL). After stirring at room temperature for 1 h, the mixture is filtered. Recrystallization from EtOH affords a yellow solid. After drying at 60°C in a vacuum oven, 4,6-diamino-2-bromo-3-cyanopyridine (12.95 g, 49%) is obtained. ^1H NMR (DMSO) δ 6.67 (2H,brs), 6.55 (2H,brs), 5.59 (1H,s).

2,4-Diamino-5-cyanopyridinium acetate. 4,6-Diamino-2-bromo-3-cyanopyridine (12.77 g, 60 mmol) is hydrogenated in THF/MeOH (240 mL, 2:1) containing KOAc (5.9 g, 60 mmol) and 20% Pd/C (0.5 g) at 18 psi at 25°C for 4 h. The mixture is celite filtered and the solvent is stripped under reduced pressure to give a solid (11.15 g) which is stirred with THF (100 mL) at room temperature for 20 min. The mixture is refiltered and the filtrate is stripped to dryness to give the desired product. After drying in a vacuum oven, 2,4-diamino-5-cyanopyridinium acetate (10.65 g, 92%) is collected as a yellow solid. ^{1}H NMR (DMSO) δ 7.90 (1H, s), 6.26 (4H, brs), 5.62 (1H, s), 1.90 (3H s).

7-Amino-4-thiono-3H-pyrido[4,3-d]pyrimidine. A mixture of 2,4-diamino-5-cyanopyridinium acetate (0.199 g, 1.0 mmol), triethyl orthoformate (1.95 mL) and Ac_2O (1.95 mL) is refluxed under N_2 with stirring for 3 h. The solvent is then stripped and the residue is dissolved in MeOH (10 mL) containing NaOMe (0.81 g, 15 mmol). H_2S is bubbled through the mixture for ~5 min, which is then refluxed overnight. After the solvent is stripped, the residue is dissolved in hot water and boiled with charcoal. After filtration, the filtrate is neutralized with acetic acid whilst hot to generate a yellow solid. On cooling, the solid is collected by suction filtration, and is dried in a vacuum oven overnight. 7-Amino-4-thiono-3H-pyrido[4,3-d]pyrimidine (84 mg, 51%) is isolated as light yellow solid. ^{1}H NMR (DMSO) δ 9.82 (1H, s), 9.34 (1H, s), 8.37 (1H, s), 7.80 (2H, d, J = 7.5 Hz), 7.38 (2H, t, J = 7.5 Hz), 7.12 (1H, t, J = 7.5 Hz), 6.61 (2H, brs) 6.43 (1H, s).

7-Amino-4-methylthiopyrido[4,3-d]pyrimidine. NEt_3 (6 mL, 43 mmol) is added to a solution of 7-amino-4-thiono-3H-pyrido[4,3-d]pyrimidine (0.77 g, 4.3 mmol) in DMSO (7 mL) stirred under N_2 at 25°C. After the two phases have been stirred for 20 min, MeI (0.26 mL, 4.2 mmol) is added. After 2 h, the reaction mixture is poured onto stirring ice-water. Solid forms instantly. After further cooling at 0°C, the solid is collected by suction filtration and dried in a vacuum oven to give 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (0.564 g, 68%). ^1H NMR (DMSO) δ 8.98 (1H, s), 8.71 (1H, s), 6.94 (2H, brs), 6.49 (1H, s) 2.63 (3H, s).

7-Amino-4-anilinopyrido[4,3-d]pyrimidine. A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (0.136 g, 0.7 mmol) and aniline (0.5 mL, 5.5 mmol) is refluxed under N_2 at 180°C for 2 h. The reaction mixture is cooled to 25°C, when it precipitates. The solid is collected by suction filtration and recrystallized from isopropanol, and dried in a vacuum oven overnight. 7-Amino-4-anilinopyrido[4,3-d]pyrimidine (84 mg, 51%) is isolated as a light yellow solid. ^1H NMR (DMSO) δ 9.82 (1H, s), 9.34 (1H, s), 8.37 (1H, s), 7.80 (2H, d, J = 7.5 Hz), 7.38 (2H, t, J = 7.5 Hz), 7.12 (1H, t, J = 7.5 Hz), 6.61 (2H, brs) 6.43 (1H, s).

Example 14

7-Amino-4-(3-hydroxyanilino)pyrido[4,3-d]pyrimidine

A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (299 mg, 1.56 mmole) and 3-aminophenol (1.60 g, 14.7 mmole) is stirred at 160 °C for 15 min. The resulting product is chromatographed over silica

gel (9% MeOH/CH_2Cl_2) to give 7-amino-4-(3-hydroxyanilino)pyrido[4,3-d]pyrimidine (108 mg, 18%) as a pale orange solid. ^{1}H NMR (DMSO) δ 9.69 (1H, brs), 9.44 (1H, brs), 9.33 (1H, s), 8.38 (1H, s), 7.37 (1H, t, J = 2.1 Hz), 7.21 (1H, brd, J = 8.4 Hz), 7.14 (1H, t, J = 8.0 Hz), 6.59 (2H, brs), 6.53 (1H, ddd, J = 7.9, 2.2, 0.8 Hz), 6.43 (1H, s).

Example 15

7-Amino-4-(3-methoxyanilino)pyrido[4,3-d]pyrimidine

A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (226 mg, 1.18 mmol) (described in the previous experimental) and *m*-anisidine (1.00 mL, 8.90 mmol) is stirred under N_2 at 190 °C for 1.5 h. The resulting product is chromatographed over silica gel (5-7% EtOH/EtOAc) to give 7-amino-4-(3-methoxyanilino)pyrido[4,3-d]pyrimidine (136 mg, 43%) as a light brown solid. ^{1}H NMR (DMSO) δ 9.78 (1H, brs), 9.34 (1H, s), 8.40 (1H, s), 7.50 (1H, brs), 7.44 (1H, d, J = 8.0 Hz), 7.28 (1H, t, J = 8.2 Hz), 6.71 (1H, dd, J = 8.2, 2.3 Hz), 6.61 (2H, brs), 6.45 (1H, s), 3.77 (3H, s).

Example 16

7-Amino-4-(2-methoxyanilino)pyrido[4,3-d]pyrimidine

A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (227 mg, 1.18 mmole) and *o*-anisidine (1.00 mL, 8.87 mmol) is stirred under N_2 at 180 °C for 2.5 h. The resulting product is chromatographed over silica gel (5% EtOH/EtOAc) to give 7-amino-4-(2-methoxyanilino)pyrido[4,3-d]pyrimidine (147 mg, 47%) as a yellow solid. ^{1}H NMR (DMSO) δ 9.44 (1H, brs), 9.25

(1H, s), 8.22 (1H, s), 7.54 (1H, dd, J = 7.7, 1.4 Hz), 7.24 (1H, ddd, J = 8.1, 7.4, 1.5 Hz), 7.10 (1H, dd, J = 8.2, 1.2 Hz), 6.98 (1H, dt, J_d = 1.3 Hz, J_t = 7.5 Hz), 6.52 (2H, brs), 6.41 (1H, s), 3.79 (3H, s).

Example 17

7-Amino-4-(3-aminoanilino)pyrido[4,3-d]pyrimidine

A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (307 mg, 1.60 mmol) (described in a previous experimental) and 3-nitroaniline (2.00 g, 14.5 mmol) is stirred at 200 °C for 1.5 h, and the crude product is suspended in MeOH/THF (4:1, 250 mL) and hydrogenated over 5% Pd/C (2 g) at 60 psi and 20 °C for 24 h. The solution is filtered over celite, washing thoroughly (hot MeOH), and is then absorbed onto alumina and chromatographed on alumina (4-8% EtOH/$CHCl_3$) to give 7-amino-4-(3-aminoanilino)pyrido[4,3-d]pyrimidine (66 mg, 16%) as a green solid,. ^{1}H NMR (DMSO) δ 9.57 (1H, brs), 9.30 (1H, s), 8.33 (1H, s), 7.04 (1H, t, J = 2.0 Hz), 6.99 (1H, t, J = 8.0 Hz), 6.88 (1H, brd, J = 8.0 Hz), 6.55 (2H, brs), 6.40 (1H, s), 6.34 (1H, dd, J = 7.9, 1.3 Hz), 5.10 (2H, brs).

Example 18

7-Amino-4-(4-aminoanilino)pyrido[4,3-d]pyrimidine

7-Amino-4-(4-acetamidoanilino)pyrido[4,3-d]pyrimidine. A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (138 mg, 0.72 mmole) and 4-aminoacetanilide (1.50 g, 10.0 mmole) is stirred under N_2 at 200 °C for 1 h. The resulting product is chromatographed over alumina (8-10% MeOH/CH_2Cl_2) to

give 7-amino-4-(4-acetamidoanilino)pyrido[4,3-d]pyrimidine (110 mg, 52%) as a pale yellow solid. ^{1}H NMR (DMSO) δ 9.94, 9.79 (1H, 1H, 2 brs), 9.31 (1H, s), 8.34 (1H, s), 7.69 (2H, d, J = 8.9 Hz), 7.57 (2H, d, J = 8.9 Hz), 6.57 (2H, brs), 6.43 (1H, s), 2.05 (3H, s).

7-Amino-4-(4-aminoanilino)pyrido[4,3-d]pyrimidine. A solution of 7-amino-4-(4-acetamidoanilino)pyrido[4,3-d]pyrimidine (0.30 g, 1.02 mmole) in aqueous NaOH (2 M, 10 mL) and MeOH (10 mL) is stirred at 100 °C for 7 h. The resulting product is chromatographed over alumina (3-4% EtOH/$CHCl_3$) to give 7-amino-4-(4-aminoanilino)pyrido[4,3-d]pyrimidine (86 mg, 33%) as an orange solid. ^{1}H NMR (DMSO) δ 9.58 (1H, brs), 9.24 (1H, s), 8.25 (1H, s), 7.31 (2H d, J = 8.6 Hz), 6.58 (2H, d, J = 8.6 Hz), 6.48 (2H, brs), 6.39 (1H, s), 5.00 (2H, brs).

Example 19

7-Amino-4-(3-dimethylaminoanilino)pyrido[4,3-d]pyrimidine

A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (245 mg, 1.28 mmol) (described in a previous experimental) and *N,N*-dimethyl-1,3-phenylenediamine (1.60 g, 11.8 mmol) is stirred under N_2 at 190 °C for 1 h, and the resulting product is chromatographed (twice) over alumina (3% EtOH/$CHCl_3$) to give 7-amino-4-(3-dimethylaminoanilino)pyrido[4,3-d]pyrimidine (113 mg, 32%) as a pale yellow solid. ^{1}H NMR (DMSO) δ 9.66 (1H, brs), 9.33 (1H, s), 8.36 (1H, s), 7.22 (1H, brd, J = 7.8 Hz), 7.16 (2H, m), 6.57 (2H, brs), 6.51 (1H, ddd, J = 8.0, 2.3, 1.2 Hz), 6.42 (1H, s), 2.91 (6H, s).

Example 20

7-Amino-4-(4-dimethylaminoanilino)pyrido[4,3-d]pyrimidine

A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (256 mg, 1.33 mmole) and *N,N*-dimethyl-1,4-phenylenediamine (1.95 g, 14.4 mmole) is stirred under N_2 at 190 °C for 20 min. The resulting product is chromatographed over alumina (3-7% EtOH/$CHCl_3$) to give 7-amino-4-(4-dimethylaminoanilino)pyrido[4,3-d]pyrimidine (198 mg, 53%) as an orange solid. ^{1}H NMR (DMSO) δ 9.67 (1H, brs), 9.27 (1H, s), 8.27 (1H, s), 7.51 (2H, d, J = 8.9 Hz), 6.75 (2H, d, J = 8.9 Hz), 6.51 (2H, brs), 6.39 (1H, s), 2.89 (6H, s).

Example 21

7-Amino-4-(2-nitroanilino)pyrido[4,3-d]pyrimidine

A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (220 mg, 1.15 mmole) and 2-nitroaniline (2.00 g, 14.5 mmole) is heated to 100 °C, then excess dry HCl gas is added to the hot stirred solution, and the mixture stirred at 160 °C for 20 min. The resulting product is neutralized with excess $NaHCO_3$, dissolved in MeOH/$CHCl_3$, dried onto silica gel and chromatographed over silica gel (2-4% MeOH/CH_2Cl_2) to give 7-amino-4-(2-nitroanilino)pyrido[4,3-d]pyrimidine (108 mg, 33%) as a yellow brown solid. ^{1}H NMR (DMSO) δ 10.40 (1H, brs), 9.24 (1H, brs), 8.20 (1H, brs), 8.12 (1H, brs), 8.01 (2H, brs), 7.75 (1H, brs), 6.70 (2H, brs), 6.43 (1H, brs).

Example 22

7-Amino-4-(3-nitroanilino)pyrido[4,3-d]pyrimidine

A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (127 mg, 0.66 mmol) (described in a previous experimental) and 3-nitroaniline (1.70 g, 12.3 mmol) is stirred under N_2 at 200°C for 1.5 h. The resulting product is chromatographed over alumina (5-20% EtOH/$CHCl_3$) to give 7-amino-4-(3-nitroanilino)pyrido[4,3-d]pyrimidine (81 mg, 39%) as a brown solid. ^1H NMR (DMSO) δ 10.17 (1H, brs), 9.37 (1H, s), 8.87 (1H, brs), 8.48 (1H, s), 8.33 (1H, brd, J = 7.5 Hz), 7.95 (1H, ddd, J = 8.2, 2.1, 1.0 Hz), 7.67 (1H, t, J = 8.2 Hz), 6.70 (2H, brs), 6.47 (1H, s).

Example 23

7-Amino-4-(3-fluoroanilino)pyrido[4,3-d]pyrimidine

A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (215 mg, 1.12 mmol) and 3-fluoroaniline (1.16 g, 10.4 mmol) is stirred at 160 °C for 30 min. The resulting product is chromatographed over silica gel (6-7% MeOH/CH_2Cl_2) to give 7-amino-4-(3-fluoroanilino)pyrido[4,3-d]pyrimidine (185 mg, 65%) as a white solid. ^1H NMR (DMSO) δ 9.94 (1H, brs), 9.36 (1H, s), 8.46 (1H, s), 7.91 (1H, brd, J = 11.9 Hz), 7.63 (1H, brd, J = 8.1 Hz), 7.41 (1H, dd, J = 15.7, 7.7 Hz), 6.93 (1H, dt, J_t = 8.5 Hz, J_d = 2.4 Hz), 6.68 (2H, brs), 6.38 (1H, s).

Example 24

7-Amino-4-(3-chloroanilino)pyrido[4,3-d]pyrimidine

A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (208 mg, 1.08 mmol) and 3-chloroaniline (1.21 g, 9.48 mmol) is stirred at 150 °C for 20 min. The resulting product is chromatographed over alumina (5-10% MeOH/CH_2Cl_2) to give 7-amino-4-(3-chloroanilino)pyrido[4,3-d]pyrimidine (177 mg, 60%) as a white solid. ^1H NMR (DMSO) δ 9.92 (1H,brs), 9.35 (1H, s), 8.45 (1H, s), 8.08 (1H, brs), 7.79 (1H, brd, J = 8.0 Hz), 7.40 (1H, t, J = 8.1 Hz), 7.16 (1H, dd, J = 7.9, 1.3 Hz), 6.68 (2H, brs), 6.46 (1H, s).

Example 25

7-Amino-4-(3,4-dichloroanilino)pyrido[4,3-d]pyrimidine

A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (247 mg, 1.29 mmol) and 3,4-dichloroaniline (1.50 g, 9.26 mmol) is stirred at 165 °C for 30 min. The resulting product is chromatographed over silica gel (7-8% MeOH/CH_2Cl_2) to give 7-amino-4-(3,4-dichloroanilino)pyrido[4,3-d]pyrimidine (252 mg, 64%) as a pale yellow solid. ^1H NMR (DMSO) δ 9.97 (1H, brs), 9.34 (1H, s), 8.47 (1H, s), 8.29 (1H, brs), 7.86 (1H, brd, J = 8.6 Hz), 7.62 (1H, d, J = 8.8 Hz), 6.70 (2H, brs), 6.46 (1H, s).

Example 26

7-Amino-4-(2-bromoanilino)pyrido[4,3-d]pyrimidine

A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (198 mg, 1.03 mmol) (described in a previous experimental) and 2-bromoaniline (1.00 mL,

9.18 mmol) is stirred under N_2 at 180°C for 2.5 h, and the resulting product is chromatographed on alumina (1% EtOH/$CHCl_3$) to give 7-amino-4-(2-bromoanilino)pyrido[4,3-d]pyrimidine (108 mg, 33%) as a pale yellow solid, ^1H NMR (DMSO) δ 9.91 (1H, brs), 9.27 (1H, s), 8.20 (1H, s), 7.73 (1H, d, J = 7.9 Hz), 7.50 (1H, m), 7.44 (1H, t, J = 6.9 Hz), 7.25 (1H, m), 6.59 (2H, brs), 6.42 (1H, s).

Example 27

7-Amino-4-(3-bromoanilino)pyrido[4,3-d]pyrimidine

A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (167 mg, 0.87 mmol) (described in a previous experimental) and 3-bromoaniline (0.75 mL, 7.8 mmol) is stirred under N_2 at 190°C for 2.5 h, and the precipitate which appears on cooling is recrystallized from Pr^iOH. ^1H NMR (DMSO) δ 9.91 (1H, brs), 9.34 (1H, s), 8.45 (1H, s), 8.19 (1H, s), 7.84 (1H, d, J = 8.0 Hz), 7.34 (1H, t, J = 8.0 Hz), 7.29 (1H, d, J = 8.2 Hz), 6.68 (2H, brs), 6.45 (1H, s).

Example 28

7-Amino-4-(4-bromoanilino)pyrido[4,3-d]pyrimidine

A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (261 mg, 1.36 mmole) and 4-bromoaniline (1.00 g, 5.81 mmole) is stirred under N_2 at 200 °C for 15 min. The resulting product is chromatographed on silica gel (10-15% EtOH/EtOAc) to give 7-amino-4-(4-bromoanilino)pyrido[4,3-d]pyrimidine (200 mg, 46%) as a pale yellow solid. ^1H NMR (DMSO) δ 9.88 (1H, brs), 9.34 (1H, s), 8.40 (1H, s), 7.83 (2H, d, J = 8.8 Hz),

7.55 (2H, d, J = 8.8 Hz), 6.64 (2H, brs), 6.44 (1H, s).

Example 29

7-Amino-4-(3-iodoanilino)pyrido[4,3-d]pyrimidine

A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (72 mg, 0.37 mmol) and 3-iodoaniline (1.25 g, 5.71 mmol) is stirred at 160 °C for 30 min. The resulting product is chromatographed over silica gel (5-7% MeOH/CH_2Cl_2) to give 7-amino-4-(3-iodoanilino)pyrido[4,3-d]pyrimidine (83 mg, 61%) as a light brown rosettes. ^1H NMR (DMSO) δ 9.84 (1H, brs), 9.34 (1H, s), 8.44 (1H, s), 8.30 (1H, brs), 7.90 (1H, dd, J = 7.9, 0.8 Hz), 7.47 (1H, d, J = 7.7 Hz), 7.18 (1H, t, J = 8.0 Hz), 6.66 (2H, brs), 6.46 (1H, s).

Example 30

7-Amino-4-(2-trifluoromethylanilino)pyrido[4,3-d]pyrimidine

A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (300 mg, 1.56 mmol), 2-aminobenzotrifluoride hydrochloride (1.00 g, 5.06 mmol) and 2-aminobenzotrifluoride (2.00 g, 12.4 mmol) is stirred at 160 °C for 10 min. The resulting product is neutralized with excess $NaHCO_3$, dissolved in MeOH/$CHCl_3$, dried onto silica gel and chromatographed over silica gel (6-7% MeOH/CH_2Cl_2) to give 7-amino-4-(2-trifluoromethylanilino)pyrido[4,3-d]pyrimidine (194 mg, 41%) as a cream solid, mp (MeOH/$CHCl_3$/light petroleum) 126-130 °C (dec.). ^1H NMR (DMSO) δ 10.60 (1H, brs), 9.17 (1H, brs), 8.13 (1H, brs), 7.76, 7.69 (1H, 1H, m, m), 7.45 (2H, m), 6.66 (2H, brs), 6.36 (1H, s).

Example 31

7-Amino-4-(3-trifluoromethylanilino)pyrido[4,3-d]pyrimidine

A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (234 mg, 1.22 mmol) (described in a previous experimental) and 3-aminobenzotrifluoride (2.00 mL, 16.0 mmol) is stirred under N_2 at 190-200°C for 2 h, and the resulting product is then chromatographed over silica gel (5-10% EtOH/EtOAc), and then over alumina (5-7% EtOH/$CHCl_3$) to give 7-amino-4-(3-trifluoromethylanilino)pyrido[4,3-d]pyrimidine (157 mg, 42%) as a cream solid. ^{1}H NMR (DMSO) δ 10.04 (1H, s), 9.37 (1H, s), 8.46 (1H, s), 8.31 (1H, s), 8.19 (1H, d, J = 8.2 Hz), 7.62 (1H, t, J = 8.0 Hz), 7.45 (1H, d, J = 7.7 Hz), 6.69 (2H, brs), 6.47 (1H, s).

Example 32

7-Amino-4-(4-trifluoromethylanilino)pyrido[4,3-d]pyrimidine

A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (390 mg, 2.03 mmol), 4-aminobenzotrifluoride hydrochloride (0.40 g, 2.02 mmol) and 4-aminobenzotrifluoride (1.61 g, 10.0 mmol) is stirred at 180 °C for 2 min. The resulting product is neutralized with excess $NaHCO_3$, dissolved in MeOH/$CHCl_3$, dried onto alumina and chromatographed over alumina (4-7% MeOH/CH_2Cl_2) to give 7-amino-4-(4-trifluoromethylanilino)pyrido[4,3-d]pyrimidine (390 mg, 63%) as a cream solid. Analytically pure material was obtained by further chromatography over silica gel (5% MeOH/CH_2Cl_2) to give pale yellow needles. ^{1}H NMR (DMSO) δ 10.09 (1H, brs), 9.40 (1H, s), 8.48 (1H, s), 8.13 (2H, d, J = 8.2 Hz), 7.74 (2H, d, J = 8.7 Hz), 6.72 (2H, brs), 6.40 (1H, s).

Example 33

4-(3-Bromoanilino)-7-methylaminopyrido[4,3-d]pyrimidine

A mixture of 7-fluoro-4-(3-bromoanilino)-pyrido[4,3-d]pyrimidine (74 mg, 0.23 mmol), triethylamine (7 mL, 50 mmol) and methylamine hydrochloride (3.0 g, 44 mmol) in isopropanol (30 mL) contained in a steel bomb is stirred at 95 °C (oil bath) for 5 h. The resulting mixture is concentrated under vacuum, basified with aqueous Na_2CO_3, diluted with water and extracted with EtOAc (3 x 100 mL). Chromatography of this extract on silica gel (3 % MeOH/CH_2Cl_2) gives 4-(3-bromoanilino)-7-methylaminopyrido[4,3-d]pyrimidine (50 mg, 65%) as a pale yellow solid. ^{1}H NMR (DMSO) δ 9.93 (1H, brs), 9.37 (1H, s), 8.47 (1H, s), 8.18 (1H, s), 7.84 (1H, d, J = 7.8 Hz), 7.34 (1H, t, J = 7.9 Hz), 7.30 (1H, brd, J = 8.1 Hz), 7.19 (1H, q, J = 4.7 Hz), 6.35 (1H, s), 2.85 (3H, d, J = 4.8 Hz).

Example 34

4-(3-Bromoanilino)-7-dimethylaminopyrido[4,3-d]pyrimidine

A mixture of 7-fluoro-4-(3-bromoanilino)pyrido[4,3-d]pyrimidine (101 mg, 0.32 mmol), triethylamine (4.4 mL, 32 mmole) and dimethylamine hydrochloride (2.58 g, 32 mmol) in isopropanol (30 mL) contained in a steel bomb is stirred at 100 °C (oil bath) for 4 h. The resulting solution is concentrated under vacuum, basified with aqueous Na_2CO_3 and diluted with water to give a solid. Filtration and recrystallisation from MeOH/$CHCl_3$ gives 7-dimethylamino-4-(3-bromoanilino)pyrido[4,3-d]pyrimidine (102 mg, 94%) as a pale yellow solid. ^{1}H NMR (DMSO) δ 9.93 (1H, brs), 9.42 (1H, s), 8.48 (1H, s), 8.19 (1H, s), 7.85 (1H, d, J = 7.7 Hz), 7.35 (1H,

t, J = 7.9 Hz), 7.30 (1H, brd, J = 7.8 Hz), 6.53 (1H, s), 3.16 (6H, s).

Example 35

4-[N-(3-Bromophenyl)-N-methylamino]-7-methylamino-pyrido[4,3-d]pyrimidine

A mixture of 7-fluoro-4-(3-bromoanilino)-pyrido[4,3-d]pyrimidine (100 mg, 0.31 mmole), triethylamine (4.4 mL, 32 mmole) and methylamine hydrochloride (2.12 g, 32 mmole) in isopropanol (30 mL) contained in a steel bomb is stirred at 100 °C (oil bath) for 5 h. The resulting mixture is concentrated under vacuum, basified with aqueous Na_2CO_3, diluted with water and extracted with EtOAc (3 x 100 mL). Chromatography of this extract on silica gel (1-2% MeOH/CH_2Cl_2) gives 4-[N-(3-bromophenyl)-N-methylamino]-7-methylaminopyrido[4,3-d]pyrimidine (23 mg, 21%) as a pale yellow solid. ^{1}H NMR (DMSO) δ 8.14 (1H, s), 7.79 (1H, s), 7.30 (1H, t, J = 8.0 Hz), 7.20 (1H, ddd, J = 7.9, 1.8, 0.8 Hz), 7.03 (1H, brq, J = 4.9 Hz), 7.01 (1H, t, J = 1.9 Hz), 6.82 (1H, ddd, J = 7.8, 1.8, 0.9 Hz), 6.25 (1H, s), 3.40 (3H, s), 2.73 (3H, d, J = 4.9 Hz).

Example 36

7-Acetylamino-4-(3-bromoanilino)pyrido[4,3-d]pyrimidine

A mixture of 7-amino-4-(3-bromoanilino)-pyrido[4,3-d]pyrimidine (0.154 g, 0.49 mmol), acetic anhydride (0.14 mL, 1.5 mmol), triethylamine (0.14 mL, 1.0 mmol) and a catalytic amount of 4-(N,N-dimethylamino)pyridine are stirred under N_2 at room temperature for 18 h. The reaction is then quenched by addition of ice water. The dark precipitate is collected by

Buchner filtration and is purified by preparative tlc (Rf = 0.25, 7% MeOH/$CHCl_3$). Recrystallization from EtOH gives 7-acetylamino-4-(3-bromoanilino)pyrido[4,3-d]-pyrimidine (13.5 mg, 7.7%). ^1H NMR (DMSO) δ 10.92 (1H, s), 10.22 (1H, s), 9.64 (1H, s), 8.70 (1H, s), 8.28 (1H, s), 8.21 (1H,s), 7.88 (1H, d, J = 7.7Hz) 7.41-7.34 (3H, m), 2.16 (3H, s).

Example 37

4-(3-Bromoanilino)-7-methoxypyrido[4,3-d]pyrimidine

A solution of 7-fluoro-4-(3-bromoanilino)-pyrido[4,3-d]pyrimidine (100 mg, 0.31 mmol) in 1 M sodium methoxide-methanol (30 mL) is stirred under reflux for 42 h. The resulting mixture is concentrated under reduced pressure, diluted with water and neutralized with dilute HCl to give 7-methoxy-4-(3-bromoanilino)pyrido[4,3-d]pyrimidine (92 mg, 89%) as a white solid. ^1H NMR (DMSO) δ 10.22 (1H, brs), 9.57 (1H, s), 8.63 (1H, s), 8.19 (1H, s), 7.86 (1H, brd, J = 7.9 Hz), 7.39 (1H, t, ^1J = 7.9 Hz), 7.35 (1H, dd, J = 7.9, 1.5 Hz), 6.96 (1H, s), 4.00 (3H, s).

Example 38

4-Benzylaminopyrido[4,3-d]pyrimidine

4-Methylthiopyrido[4,3-d]pyrimidine (160.4 mg, 0.902 mmol), and benzylamine (106.3 mg, 0.992 mmol) in EtOH (2 mL) are heated at 80°C for 12 h, and then the solvent is removed under reduced pressure. The resulting solid is suspended in CH_2Cl_2, filtered, and the resulting solid is purified by preparative tlc on silica, eluting with 5% MeOH in $CHCl_3$. Removal of the solvent under reduced pressure yields 4-

benzylaminopyrido[4,3-d]pyrimidine (36 mg, 17%). ^{1}H NMR (DMSO) δ 9.60 (1H, s), 9.37 (1H, t, J = 5.8 Hz), 8.72 (1H, d, J = 5.8 Hz), 8.57 (1H, s), 7.54 (1H, d, J = 5.8 Hz), 7.37 (2H, d, J = 7.0 Hz), 7.33 (2H, t, J = 7.3 Hz), 7.25 (1H, t, J=7.2 Hz), 4.81 (2H, d, J = 5.8 Hz).

Example 39

4-([R]-1-Phenylethylamino)pyrido[4,3-d]pyrimidine

To a mixture of 4-methylthiopyrido[4,3-d] pyrimidine (85 mg, 0.48 mmol) and EtOH (2.5 mL) is added R-methylbenzylamine (0.13 mL, 1.0 mmol) dropwise. The resulting mixture is refluxed at 80°C for 20 h. The solvent is removed under reduced pressure to give an oil which is crystallized from MeOH to give 4-([R]-1-phenylethylamino)pyrido[4,3-d]pyrimidine (41.6 mg, 35%), mp 138-138.5°C. ^{1}H NMR (DMSO) δ 9.77 (1H, d, J = 0.7 Hz), 9.00 (1H, d, J = 7.7 Hz), 8.73 (1H, d, J = 5.8 Hz), 8.54 (1H, s), 7.53 (1H, dd, J = 5.8, 0.5 Hz), 7.45 (2H, d, J = 7.2 Hz), 7.33 (2H, t, J = 7.6 Hz), 7.23 (1H, tt, J = 7.5, 1.2 Hz), 5.63 (1H, p, J = 7.2 Hz), 1.61 (3H, t, J = 7.0 Hz).

Example 40

7-Amino-4-benzylaminopyrido[4,3-d]pyrimidine

A mixture of 2,4-diamino,5-cyanopyridinium acetate (8.78 g, 45 mmol), formic acid (10.66 g, 0.204 mol) and benzylamine (45 mL, 0.41 mol) is heated at 200°C under N_2 for 2 h. Upon cooling, it solidifies. Water (500 mL) is added and the gummy solid/water mixture is stirred for ~20 min. at 0°C. The liquid is

decanted. The solid is washed with water and then recrystallized from isopropanol (25 mL). After drying in a vacuum oven overnight, 7-amino-2-benzylaminopyrido[4,3-d]pyrimidine (8.29 g, 73%) is obtained as a light yellow solid. ^{1}H NMR (DMSO) δ 9.10 (1H, s), 8.85 (1H, t, J = 5.8 Hz), 8.25 (1H, s), 7.21-7.36 (5H, m), 6.46 (2H, brs), 6.35 (1H, s), 4.74 (2H, d, J = 6.0 Hz).

Example 41

7-Amino-4-([R]-1-phenylethylamino)pyrido[4,3-d]pyrimidine

A mixture of [R]-1-phenylethylamine (0.072 mL, 0.55 mmol) and 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (97 mg, 0.5 mmol) (described in a previous experimental) is heated at 180°C under N_2 for 1.5 hr. The reaction is then cooled to room temperature producing a precipitate. The mixture is added to water and $CHCl_3$, sonicated and filtered. The phases are separated and the aqueous phase is extracted with $CHCl_3$. The combined extracts are washed with water, saturated brine and dried ($MgSO_4$). The solvent is removed under reduced pressure and the residue purified by using preparative tlc (5% MeOH/$CHCl_3$) and recrystallization from $CHCl_3$ to give 7-amino-4-([R]-1-phenylethylamino)pyrido[4,3-d]pyrimidine (14.5 mg, 11%), mp 231.8-232.1°C. ^{1}H NMR (DMSO) δ 9.23 (1H, s), 8.50 (1H, d, J = 8.0 Hz), 8.19 (1H, s), 7.41 (2H, d, J = 7.0 Hz), 7.31 (2H, t, J = 8.0 Hz, 7.21 (1H, tt, J = 7.4, 1.2 Hz), 6.45 (2H, s), 6.33 (1H, s), 5.56 (1H, p, J = 7.2 Hz), 1.55 (3H, d, J = 7.0 Hz).

Example 42

7-Amino-4-(2-aminobenzylamino)pyrido[4,3-d]pyrimidine

A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (136 mg, 0.71 mmol) (described in a previous experimental) and 2-aminobenzylamine (1.70 g, 13.8 mmol) in isopropanol (5 mL) is stirred at reflux for 1 h, and the resulting product is chromatographed on silica gel (7-20% EtOH/EtOAc) and alumina (6-10% EtOH/$CHCl_3$) to give 7-amino-4-(2-aminobenzylamino)pyrido[4,3-d]pyrimidine (89 mg, 47%) as a white solid. ^1H NMR (DMSO) δ 9.08 (1H, s), 8.68 (1H, t, J = 5.8 Hz), 8.26 (1H, s), 7.05 (1H, d, J = 7.4 Hz), 6.96 (1H, t, J = 7.6 Hz), 6.63 (1H, d, J = 7.9 Hz), 6.51 (1H, t, J = 7.4 Hz), 6.46 (2H, brs), 6.35 (1H, s), 5.20 (2 H, brs), 4.56 (2H, d, J = 5.8 Hz).

Example 43

7-Amino-4-(3-dimethylaminobenzylamino)pyrido[4,3-d]-pyrimidine

A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (236 mg, 1.23 mmol) (described in a previous experimental) and 3-dimethylamino-benzylamine (1.36 g, 9.07 mmol) in isopropanol (5 mL) is stirred under N_2 at reflux for 1 h, and the resulting product is chromatographed on silica gel (10-15% EtOH/EtOAc), then on alumina (1% EtOH/$CHCl_3$) to give 7-amino-4-(3-dimethylaminobenzylamino)pyrido[4,3-d]pyrimidine (145 mg, 40%) as a white solid. ^1H NMR (DMSO) δ 9.11 (1H, s), 8.79 (1H, t, J = 5.9 Hz), 8.26 (1H, s), 7.11 (1H, dd, J = 8.0, 7.7 Hz), 6.73 (1H, brs), 6.63 (1H, d, J = 7.6 Hz), 6.60 (1H, dd, J = 8.1, 2.2 Hz), 6.44 (2H,

brs), 6.35 (1H, s), 4.67 (2H, d, J = 5.8 Hz), 2.86 (6H, s).

Example 44

7-Amino-4-(3-nitrobenzylamino)pyrido[4,3-d]pyrimidine

A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (228 mg, 1.19 mmol) (described in a previous experimental) and 3-nitrobenzylamine (0.81 g, 5.33 mmol) is stirred under N_2 at 150-160 °C for 1.5 h, and the resulting product chromatographed on silica gel (5-10% EtOH/EtOAc) to give 7-amino-4-(3-nitrobenzylmino)pyrido[4,3-d]pyrimidine (151 mg, 43%) as a yellow solid. ^{1}H NMR (DMSO) δ 9.11 (1H, s), 8.98 (1H, t, J = 5.5 Hz), 8.26 (1H, s), 8.22 (1H, brs), 8.12 (1H, dd, J = 8.0, 1.8 Hz), 7.83 (1H, d, J = 7.7 Hz), 7.63 (1H, t, J = 7.9 Hz), 6.50 (2H, brs), 6.38 (1H, s), 4.85 (2H, d, J = 5.8 Hz).

Example 45

7-Amino-4-(3-methoxybenzylamino)pyrido[4,3-d]pyrimidine

A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (136 mg, 0.71 mmol) (described in a previous experimental) and 3-methoxybenzylamine (1.37 g, 10.0 mmol) in isopropanol (3 mL) is stirred under N_2 at reflux for 3 h. Evaporation of the solvent and chromatography on silica gel (5-10% EtOH/EtOAc) gives 7-amino-4-(3-methoxybenzylamino)pyrido[4,3-d]pyrimidine (153 mg, 77%) as a white solid. ^{1}H NMR (DMSO) δ 9.11 (1H, s), 8.83 (1H, t, J = 5.7 Hz), 8.26 (1H, s), 7.24 (1H, dt, J_d = 0.8 Hz, J_t = 8.1 Hz), 6.92 (2H, m), 6.81 (1H, dt, J_d = 8.2 Hz, J_t =1.2 Hz), 6.46 (2H, brs), 6.37 (1H, s), 4.71 (2H, d, J = 5.8 Hz), 3.73 (3H, s).

Example 46

7-Amino-4-(4-chlorobenzylamino)pyrido[4,3-d]pyrimidine mesylate

The free base (56 mg, 0.20 mmol) (prepared from 2,4-diamino,5-cyanopyridinium acetate, formic acid and 4-chlorobenzylamine at 200°C as described in a previous example is precipitated from acetone solution with methanesulfonic acid (105 μL, 0.23 mmol) to give a polymesylate salt. ^{1}H NMR (DMSO) δ 10.59 (1H, t, J = 5.6 Hz), 9.24(1H, s), 8.69 (1H, s), 7.42 (4H, s), 6.42 (1H, s), 5.8 (~6H, vbrs), 4.89 (2H, d, J = 5.8 Hz), 2.41 (~7.5H, s).

Example 47

7-Amino-4-(2-bromobenzylamino)pyrido[4,3-d]pyrimidine

A mixture of 7-amino-4-methylthiopyrido[4,3-d]pyrimidine (225 mg, 1.17 mmol) (described in a previous experimental) and 2-bromobenzylamine (0.84 g, 4.52 mmol) is stirred under N_2 at 140 °C for 1 h, and the resulting product chromatographed on silica gel (1-5% EtOH/EtOAc) to give 7-amino-4-(2-bromobenzylamino)pyrido[4,3-d]pyrimidine (175 mg, 45%) as a light brown solid. ^{1}H NMR (DMSO) δ 9.16 (1H, s), 8.85 (1H, t, J = 5.7 Hz), 8.24 (1H, s), 7.64 (1H, d, J = 7.8 Hz), 7.34 (1H, dd, J = 7.7, 7.1 Hz), 7.31 (1H, dd, J = 7.7, 2.4 Hz), 7.21 (1H, ddd, J = 7.8, 6.9, 2.4 Hz), 6.50 (2H, brs), 6.39 (1H, s), 4.74 (2H, d, J = 5.7 Hz).